1. Introduction to Cytogenetics

Introduction to cytogenetics

Janet M. Cowan, Ph.D.

2. Learning objectives

Learning objectives

- Normal karyotype: number of chromosomes, basic nomenclature
- Typical indications for chromosome analysis in the newborn and adult. Examples of the findings
- Meiosis and the errors that can lead to trisomy/mis-segregation of balanced translocations

3. How to see chromosomes

How to see chromosomes

Chromosomes only visible as cells approach and pass through metaphase in cell cycle

What is seen is DUPLICATED chromosome

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4. Normal karyotype

Normal karyotype

Normal human karyotype has 46 chromosomes - 22 pairs of autosomes and a pair of sex chromosomes

Female = 46,XX

Male = 46,XY

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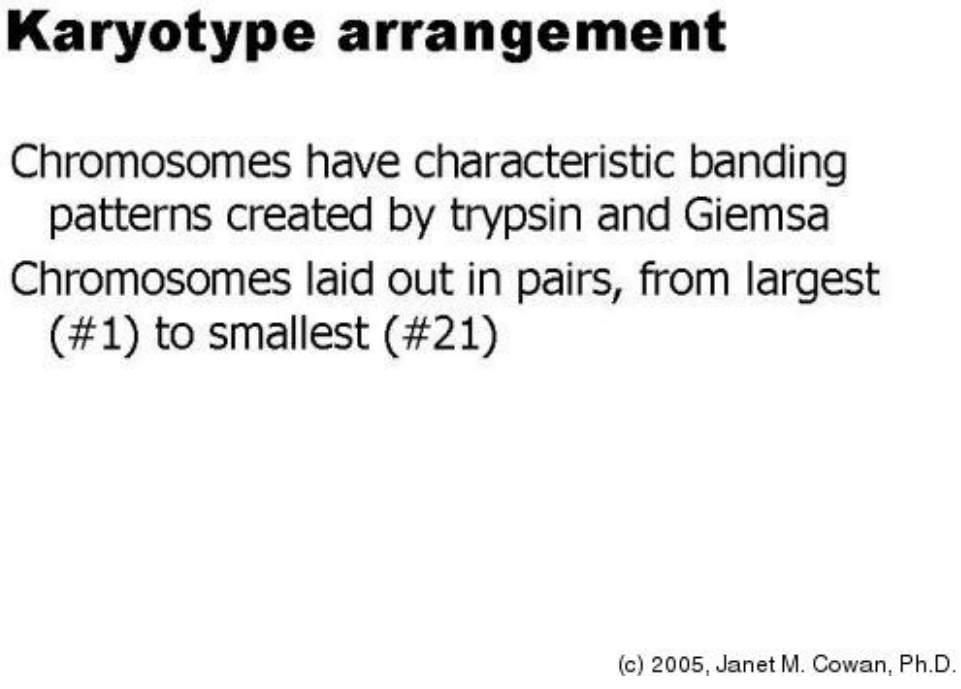
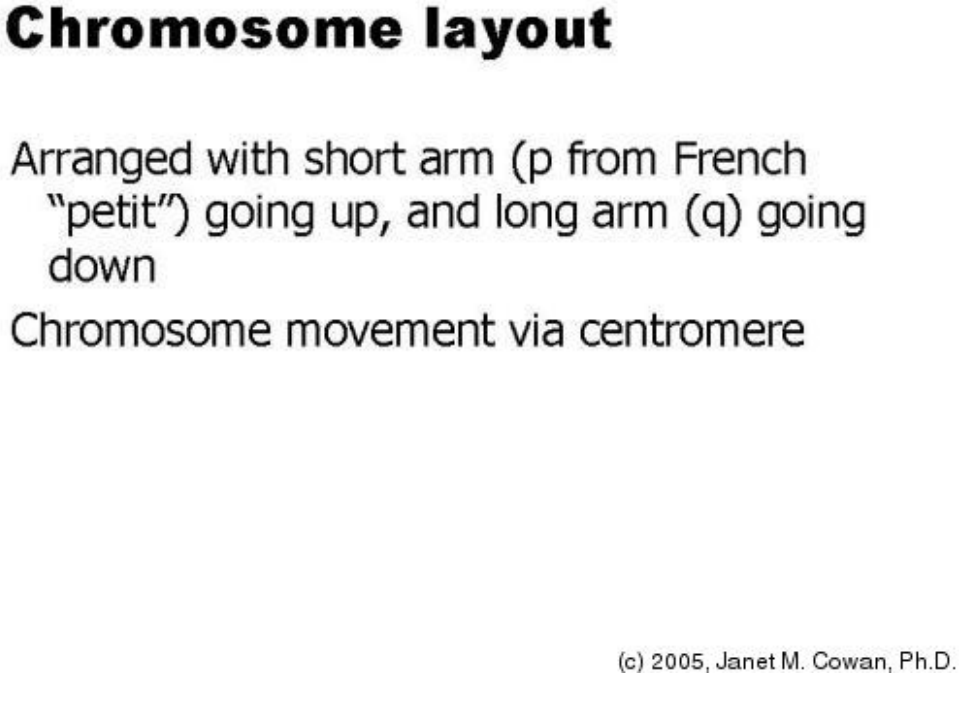
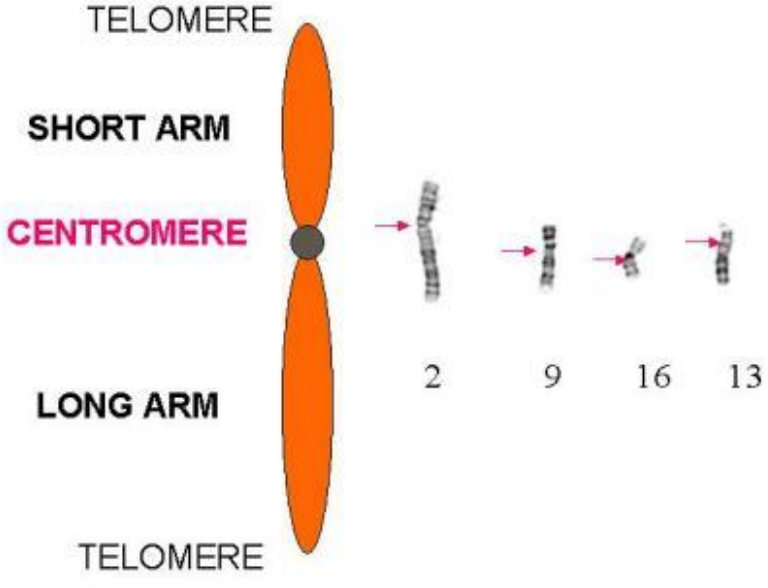
5. Karyotype arrangement



6. Chromosome layout



7. Diagram



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8. Nomenclature

Nomenclature

International System for Human Cytogenetic Nomenclature (ISCN)

Karyotype components:

Number of chromosomes,sex,first change,second change,etc.

Sex chromosome changes are listed first and then others in numerical order

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9. Abbreviations

Abbreviations

p = short arm
q = long arm
+ = additional chromosome
- = missing chromosome

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10. Abbreviations

Abbreviations

t = translocation
del = deletion
dup = duplication
inv = inversion
der = derivative = structurally rearranged chromosome

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11. Monosomy

Monosomy

Loss of one copy of a chromosome

45,X known

No other full monosomies reported

Loss of part of a chromosome = monosomy for small region

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12. Trisomy/triploidy

Trisomy/triploidy

Trisomy = three copies of a chromosome or part of a chromosome - normal chromosome number increased by 1 to 47

Triploidy = three copies of EVERY chromosome - normal chromosome number increased by 23 to 69

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13. Examples 1

Examples 1

Trisomy 21: 47,XY,+21

Trisomy 18: 47,XX,+18

Balanced translocation:
46,XX,t(3;5)(p21;q31)

Chromosomes listed in numerical order

Breakpoints listed in same order

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14. Typical newborn presentation

Typical newborn presentation

Baby G is admitted to Neonatal Intensive Care Unit. Staff note that baby has unusual features and call for Genetics consult

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15. Baby G

Baby G

Genetics find the baby has: Hypotonia, protruding tongue, slanted palpebral fissures, small nose with low nasal bridge, small ears, short neck, relatively short metacarpels and phalanges in hands, wide gap between first and second toes ("sandal gap")

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16. In the lab

In the lab

0.25 – 0.5 ml blood added to 5 ml tissue culture medium (with fetal calf serum)

Phytohemagglutinin (PHA) added to stimulate T-cells to divide

First harvest done after 48 hours

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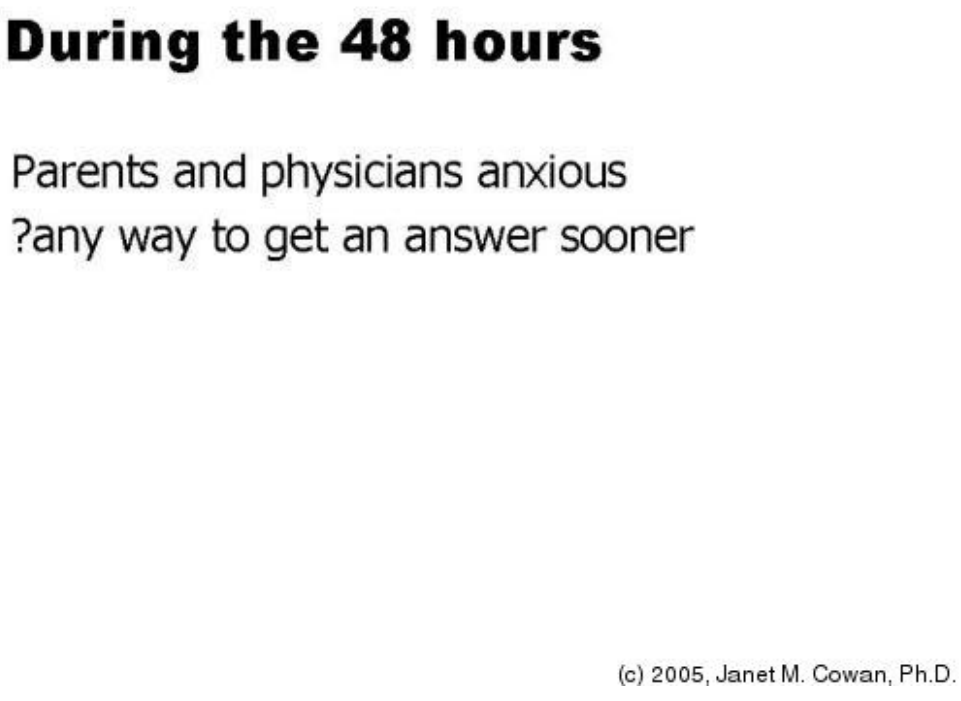
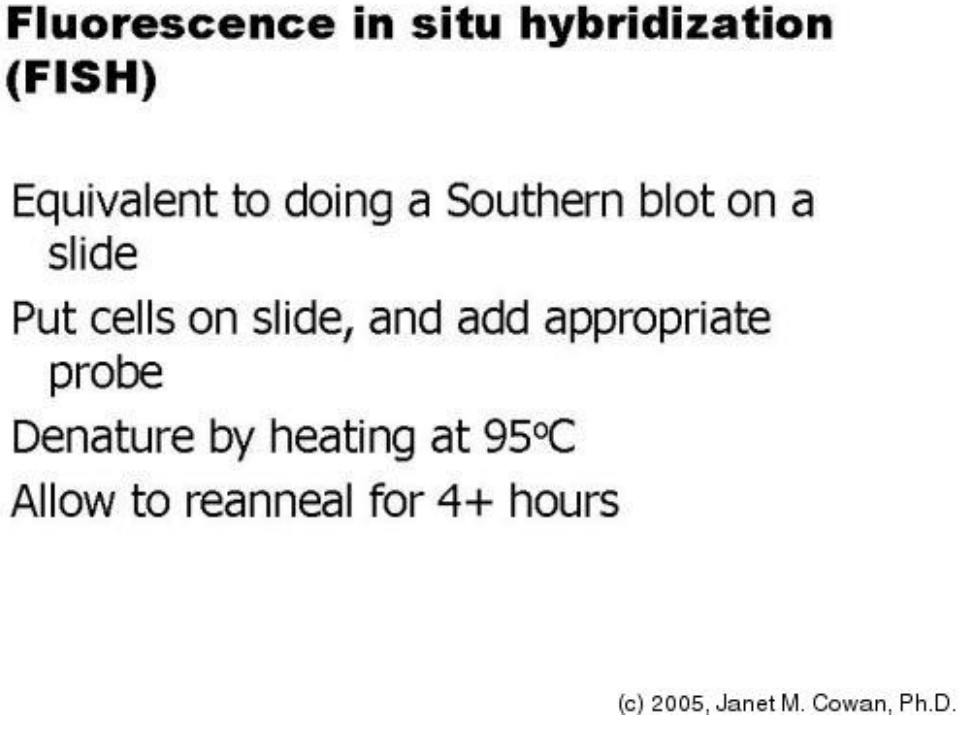
17. During the 48 hours



18. Fluorescence in situ hybridization (FISH)



19. FISH probes

FISH probes

Single copy probes – disease specific regions such as trisomy 21 critical band

Centromeric probes - alpha satellite regions of chromosomes, used to count chromosomes

Paints - made from flow-sorted chromosomes, used to confirm translocations etc.

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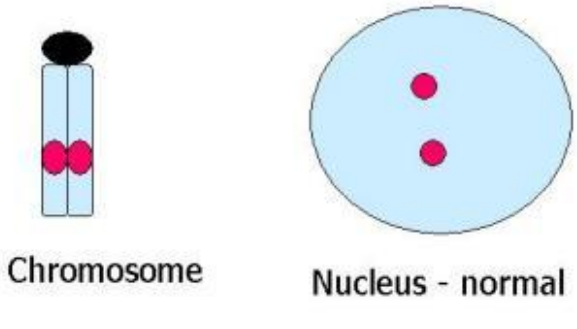
20. Chromosome 21 FISH

Chromosome 21 FISH

Use mixture of single copy probes from 21q22 (trisomy 21 critical region)



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21. Back to the blood in culture

Back to the blood in culture

Harvest:

Colchicine is added to disrupt mitotic spindle

Hypotonic solution added to swell the cells

Cells fixed

Cell suspension dropped onto slides

Slides are treated with trypsin solution and stained (G-banding)

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22. How does trisomy 21 happen?

How does trisomy 21 happen?

Gametes form during meiosis

In meiosis 1 (MI) homologous chromosomes go to different daughter cells

In meiosis 2 (MII) each chromosome divides along its length and each chromatid goes to a daughter cell

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23. Frequency of trisomy

Frequency of trisomy

Trisomy	*Frequency*	*Parental Origin*
47,XX/XY,+21	1 in 700	Mat MI>mat MII 3:1
47,XX/XY,+18	1 in 6 – 8,000	Mat MI or MII
47,XX/XY,+13	1 in 12,000	Mat MI or MII

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24. Typical routine adult presentation

Typical routine adult presentation

Mr. and Mrs. F, who are in their late 20's, are referred to a fertility clinic because they have been married for 5 years, but have no children

Mrs. F has been pregnant four times, but all pregnancies have ended in miscarriage

As part of their work up, blood is sent for karyotype analysis

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25. In the lab

In the lab

Blood is cultured as before, but for 72 hours

Mr F is found to have normal karyotype, 46,XY

Mrs F is found to have apparently balanced translocation between chromosomes 10 and 11

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26. Mrs F’s karyotype

Mrs F's karyotype

46,XX,t(10;11)(p15;q21)

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27. Translocations

Translocations

Exchange of material between two or more chromosomes

Constitutional balanced translocation usually has no effect on phenotype

May result in infertility or history of spontaneous abortion

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28. Constitutional translocations

Constitutional translocations

Present at birth in some or all of cells in body and most are unique to a family

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29. Robertsonian translocations

Robertsonian translocations

Occur between acrocentric chromosomes (13/14/15/21/22)

Results in loss of non-critical genes in the short arm regions of the chromosomes involved

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30. Robertsonian translocation

Robertsonian translocation

Count is decreased to 45:

45,XY,der(15;22)(q10;q10)

45,XX,der(13;14)(q10;q10)

"q10" means that the break is in the centromere and the long arm is present

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31. Translocations and meiosis

Translocations and meiosis

Chromosomes pair along their lengths as far as possible making a four - armed figure

Centromeres of homologs repel each other and go to different daughter cells

Segregation errors may lead to combination of monosomy and trisomy

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32. Mr and Mrs F

Mr and Mrs F

Decide to try to get pregnant one more time

Pregnancy ends in spontaneous loss

Tissue sent to the lab

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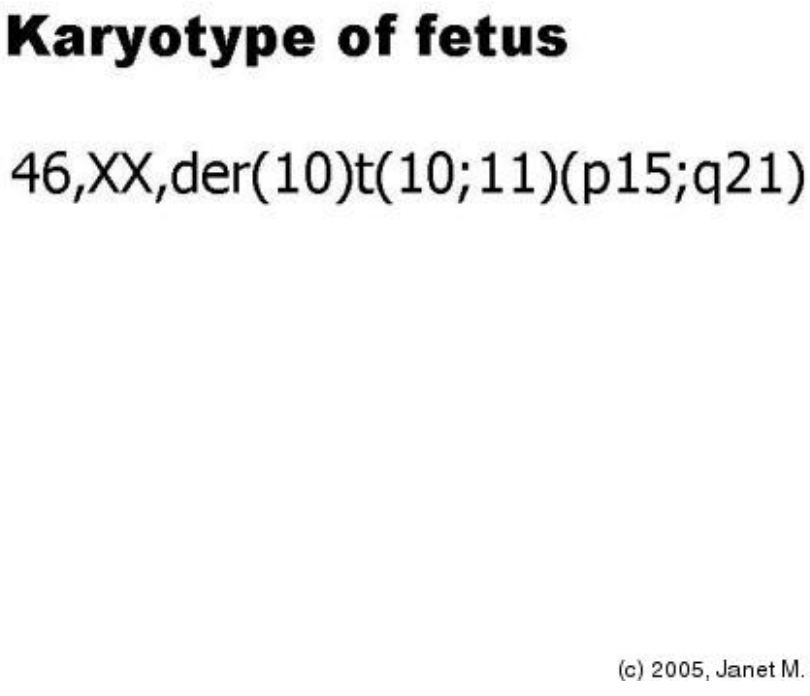
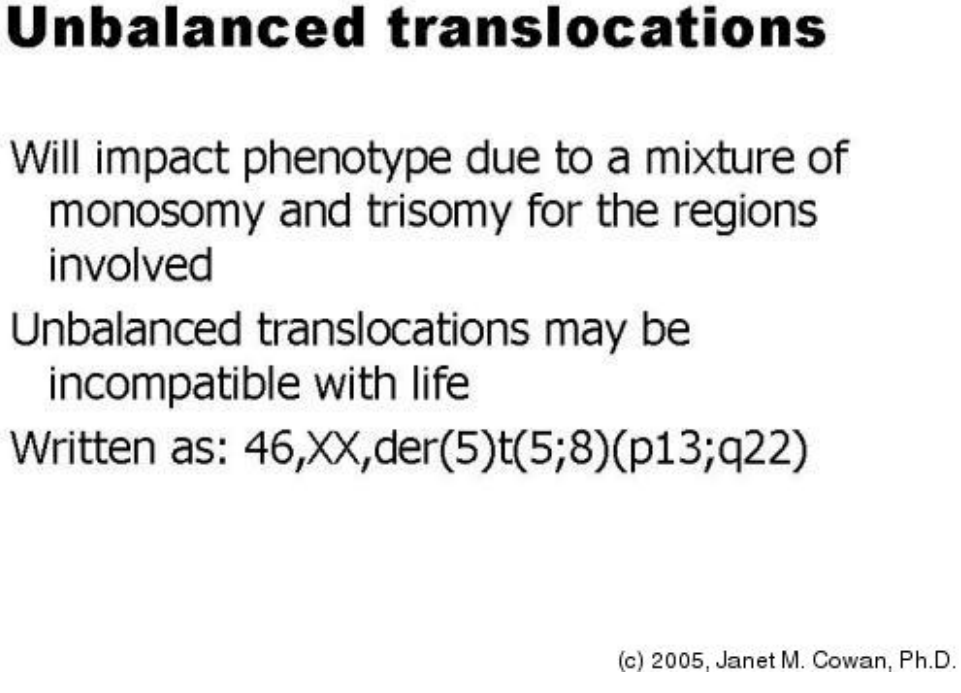
33. Karyotype of fetus



34. Unbalanced translocations



35. Summary 1

Summary 1

Normal karyotype = 46 chromosomes
- 22 pairs of chromosomes and 2 sex chromosomes (46,XX or 46,XY)

Chromosomes divided by the centromere into short (p) and long (q) arms

Each chromosome has a number of bands, each of which has been assigned a number

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36. Summary 2

Summary 2

Karyotypes written using a system of nomenclature

Abbreviations used to describe changes such as t, der, dup, inv

Balanced constitutional rearrangements (translocation or inversion) do not alter phenotype and most are unique to a family

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37. Summary 3

Summary 3

Unbalanced constitutional rearrangements result in duplication and deletion of material and are associated with changes in phenotype