Cytogenetic and Molecular Diagnosis in Gestational Disorders

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UNIVERSITY OF UTAH SCHOOL OF MEDICINE

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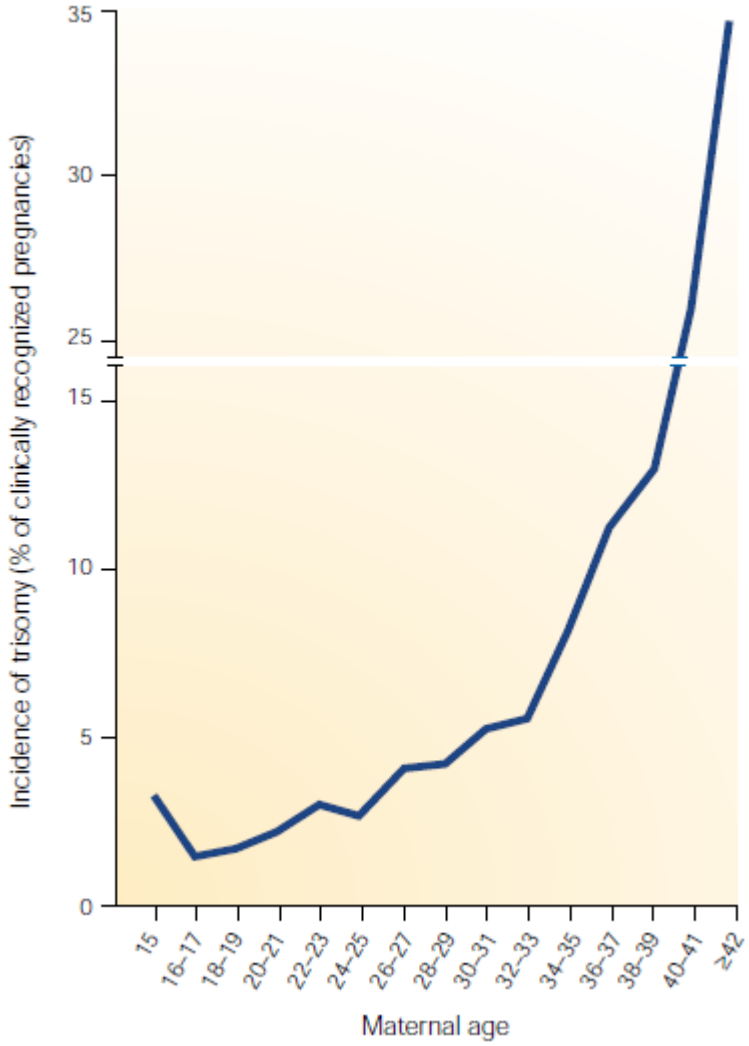
Overview

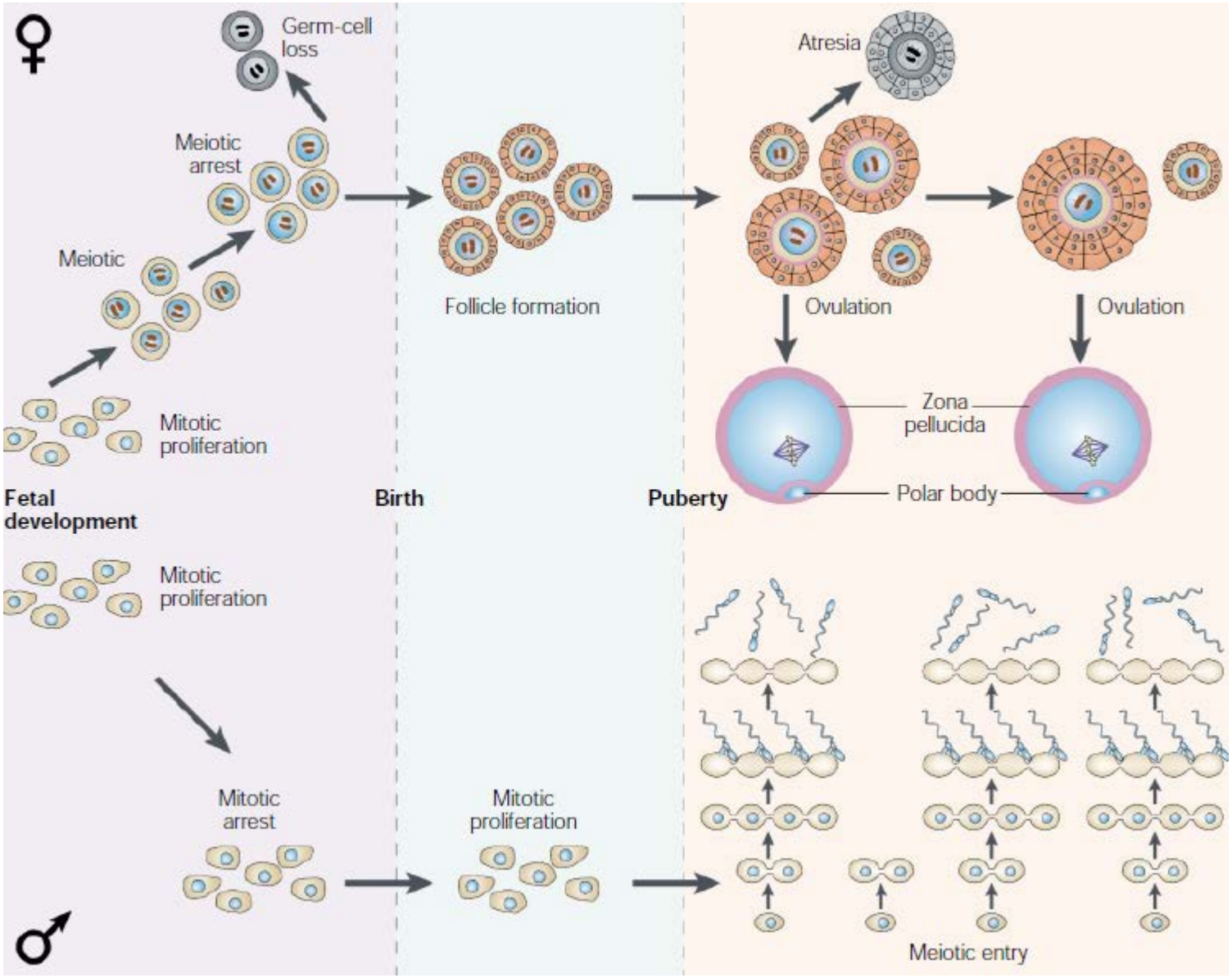
- Discuss the role of molecular genetic testing in evaluating pathologic conditions related to pregnancy
  - Viable pregnancy: prenatal screening and invasive diagnostic testing for genetic abnormalities
  - Pregnancy loss
  - Molar pregnancy
- Review laboratory testing strategies

1. PRENATAL SCREENING AND INVASIVE DIAGNOSTIC TESTING FOR GENETIC ABNORMALITIES

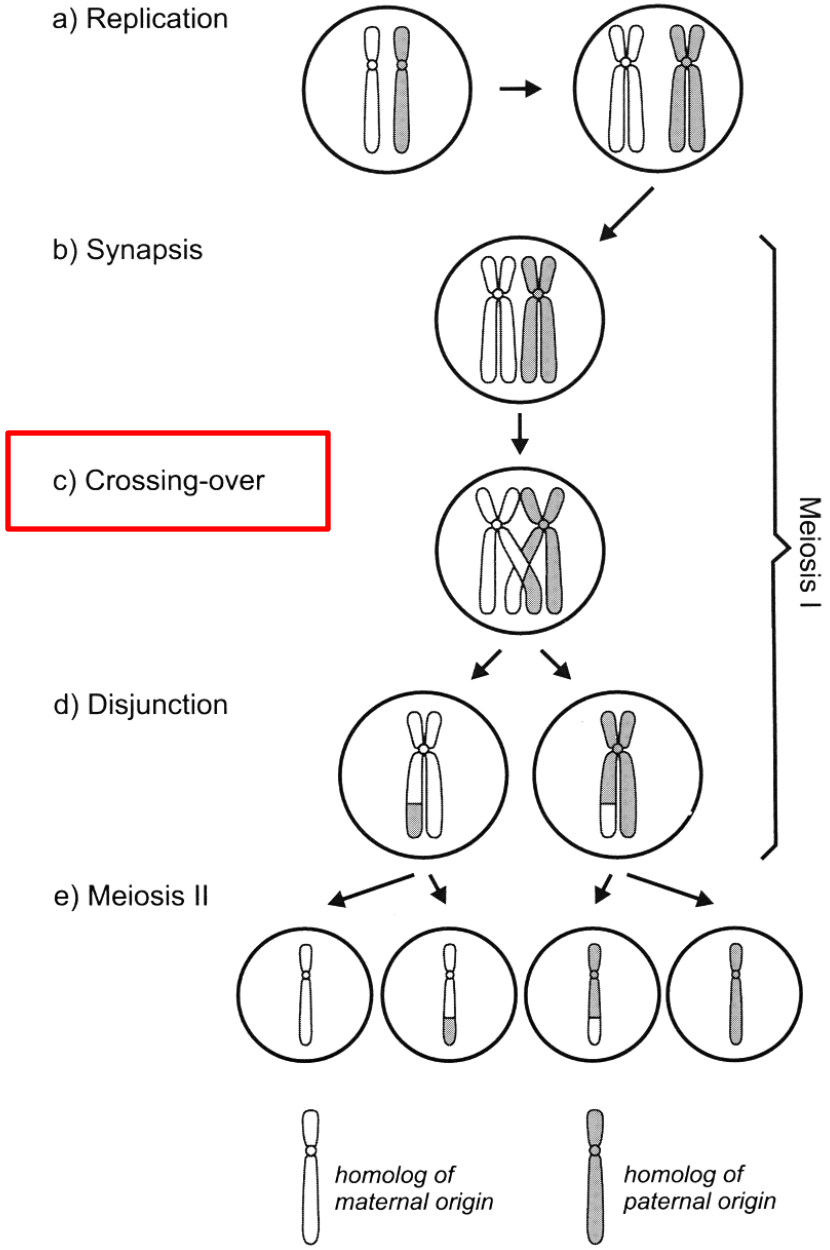
The incidence of aneusomy increases dramatically with maternal age



Maternal age and trisomy. Fig 6 in Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2001;2:280-91.



Meiotic 'timelines' for humans. Fig 1 in Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2001;2:280-91.



Meiotic errors are correlated with the <u>number</u> and <u>location</u> of recombination events

Fig. 2-2 Chromosome behavior in meiosis I. In: Gersen SL, Keagle MB. The Principles of Clinical Cytogenetics. New Jersey: Humana Press; 2005.

Screening tests for genetic abnormalities of the embryo/ fetus

- Noninvasive tests for common trisomies (21, 18, (13))
  - Ultrasound
    - First trimester – nuchal translucency
    - Second trimester – anatomy scan (18-20 weeks)
  - Maternal serum screening
    - Biochemical markers of aneuploidy (PAPP-A, beta-hCG, AFP, uE3, inhibin A)
    - Cell free fetal DNA
- Ethnicity-based (parental carrier) screening for single gene disorders
  - Cystic fibrosis (Caucasian, Ashkenazi Jewish)
  - Tay Sachs disease and others (Ashkenazi Jewish)
  - Hemoglobinopathies (Mediterranean descent)

Non-invasive prenatal testing (NIPT)

- Cell free fetal DNA
  - Short fragments of DNA circulating in maternal serum, small proportion (<10%) of total DNA
  - Detectable in maternal serum by 5th week; short half life (disappears within minutes postpartum)
- Companies currently offering NIPT (massively parallel sequencing)
  - Sequenom MaternaT21™ Plus
  - Verinata Health - verifi® Prenatal Test
  - Ariosa Diagnostics - Harmony™ Prenatal Test
- Other technologies in development
- The National Society of Genetic Counselors (NSGC) recommends genetic counseling with NIPT, and follow up of abnormal results with a conventional diagnostic procedure (amniocentesis)

Non-invasive prenatal testing (NIPT): current controversies

- Variably regarded as “diagnostic” vs. “screening” test
- Concern over direct-to-consumer marketing
- Large preclinical trials have been performed, but more clinical validation studies are needed
- Lower sensitivity and specificity for trisomy 13
- Changing landscape of prenatal genetic testing
  - No longer restricted to women > 35
  - Shift toward increased patient autonomy
- Destigmatization of Down syndrome
- State-led initiatives to restrict abortions, concern over potential increase in pregnancy termination rate

Definitive (diagnostic) tests for genetic abnormalities of the embryo/ fetus

- Direct fetal sample is obtained via invasive procedure:
  - Chorionic villus sampling (11 - 13 weeks)
  - Amniocentesis (15 – 20 weeks)
- Offered to all pregnant women, along with genetic counseling, regardless of age (ACOG Practice Bulletin No. 77, 2007)
- Preferred method of follow up for abnormal ultrasound and/ or abnormal prenatal screening (risk > 1:250 – 1:300)
- Cytogenomic testing methodologies:
  - Karyotype (chromosome analysis, cytogenetics)
    - All indications, including maternal preference for diagnostic testing
  - Genomic microarray for copy number variations, including microdeletion syndromes
    - Highest diagnostic yield (Wapner et al, *NEJM* 367 (23): 2175-84, 2012).

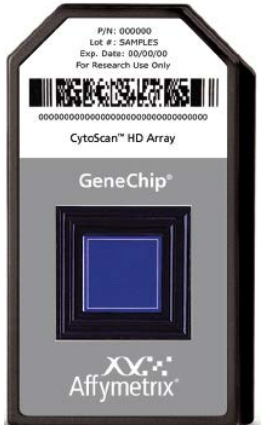
Definitive (diagnostic) tests for genetic abnormalities of the embryo/ fetus

- Targeted molecular genetic testing
  - FISH or microsatellite genotyping for aneuploidy
  - Single gene / gene panel testing for Mendelian disorders in the fetus

Chromosomal microarray

- DNA-based testing for copy number variations (CNVs) throughout the genome
- Size of detectable CNVs depends on array design and probe coverage

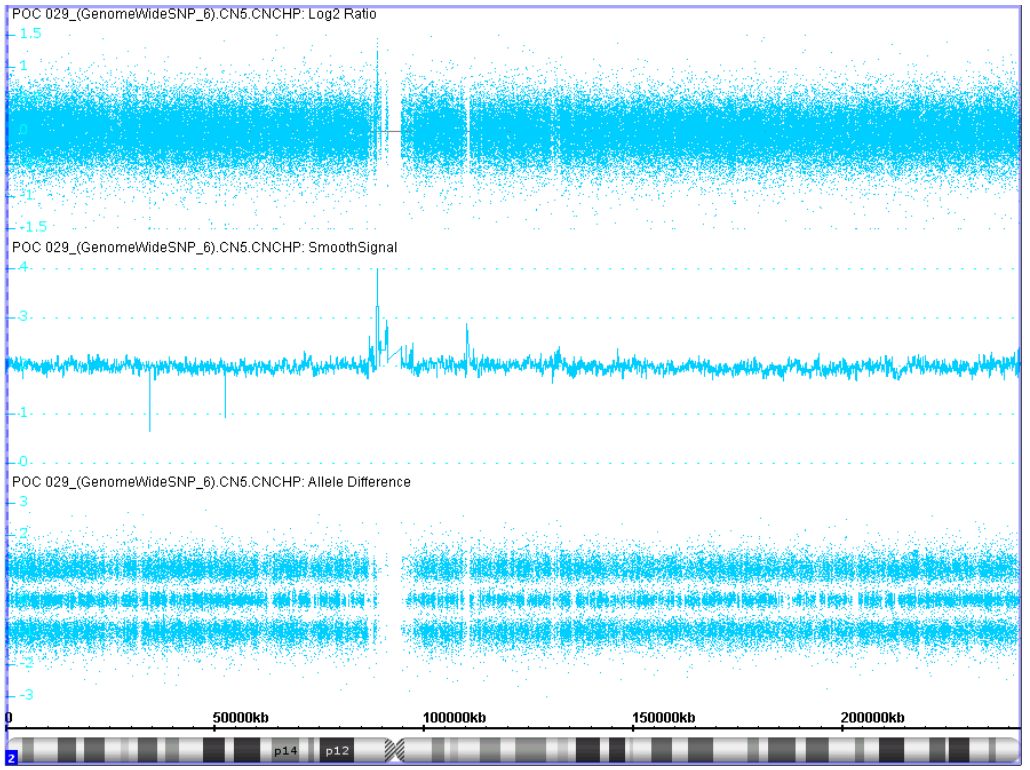
Affymetrix
CytoScan SNP array



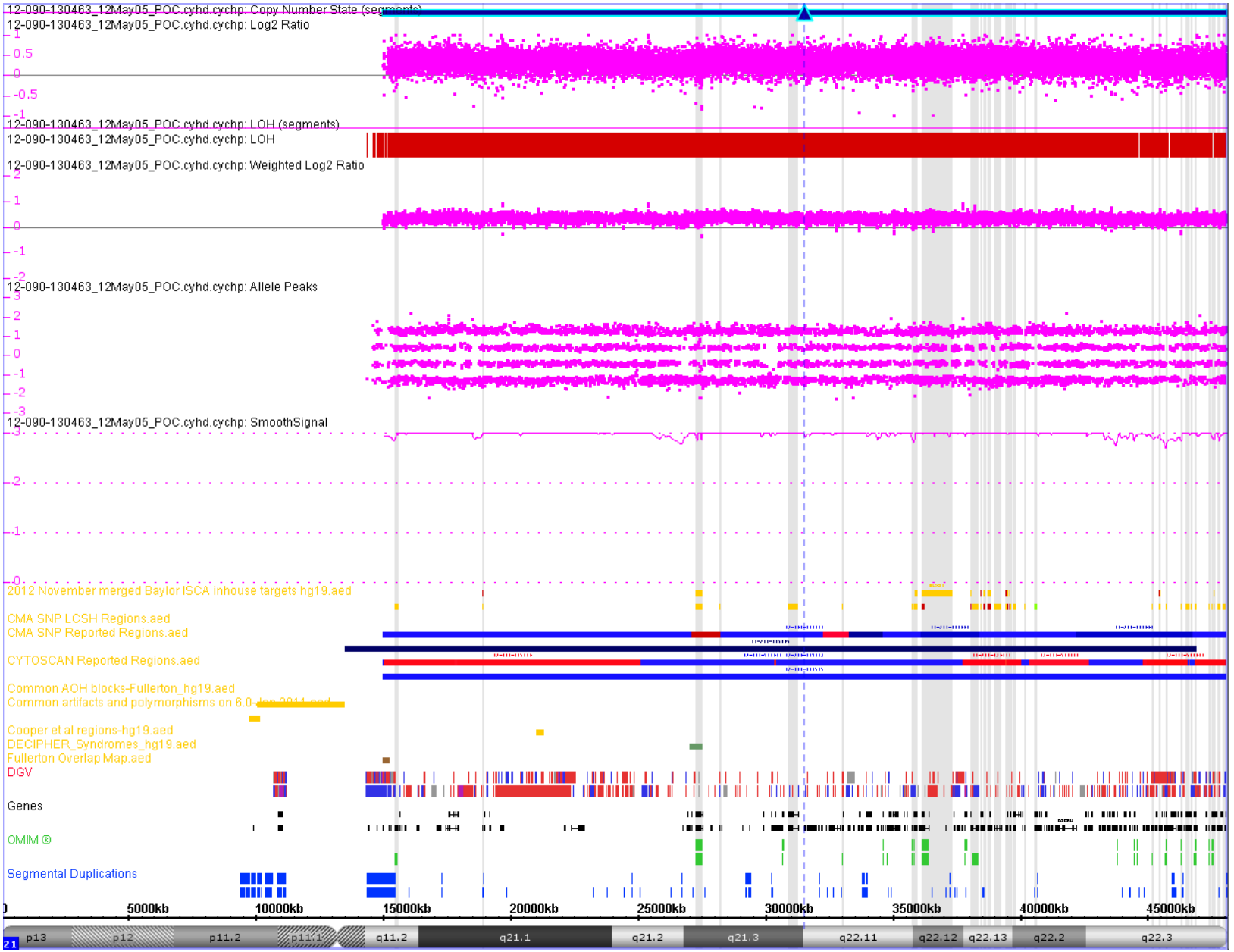
log2 ratio (copy#) →

"smooth signal" (copy#) →

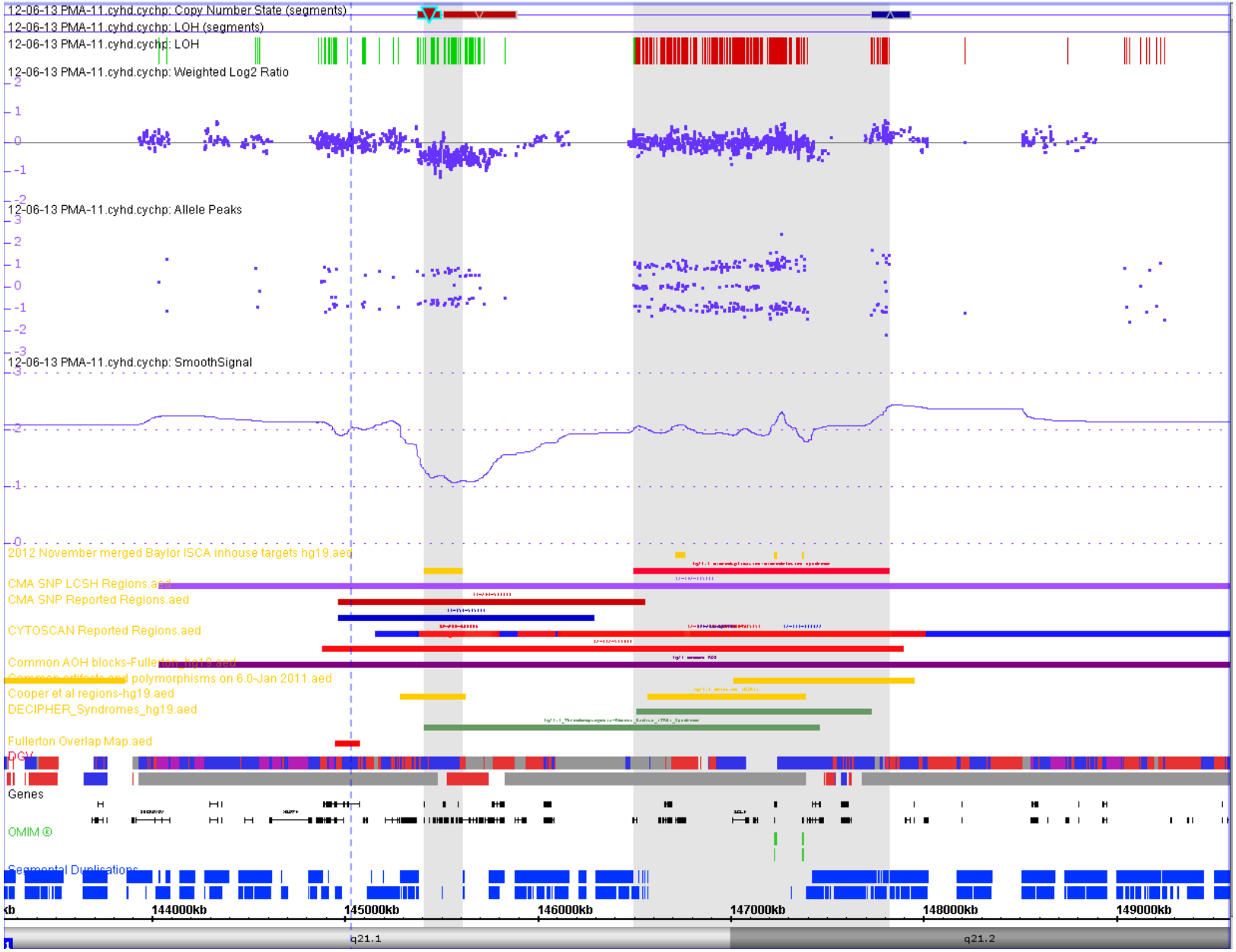
allele difference track (SNP probes) →



Microarray showing Trisomy 21



Microarray - 1q21 microdeletion (346.8 kb within 2 Mb common microdeletion region)



2. PREGNANCY LOSS

Pregnancy loss is common in humans

- 15-20% of clinically recognized pregnancies
  - Recurrent miscarriage (≥2): 1%
  - Most losses (90%) occur during the first trimester
- 30-40% of chemically detected pregnancies
  - In a 1988 *NEJM* study*, women who were trying to achieve pregnancy were monitored by daily urine beta hCG levels. A total of 31% of detected pregnancies were lost.
    - 2/3 of the lost pregnancies were undetectable clinically
- Unknown % of pre-implantation pregnancies
  - Cytogenetic studies of IVF pre-embryos at day 3 has shown that about 50% are genetically abnormal
- Probably <50% of conceptions result in live birth

*Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of Early Loss of Pregnancy. New England Journal of Medicine 1988;319:189-94.

Etiology

- An etiology for the loss can be determined in only about half of unintended pregnancy losses

- Genetic abnormality
- Immunologic factors
- Infections
- Endocrine
- Environmental agents
- Uterine anatomic abnormalities

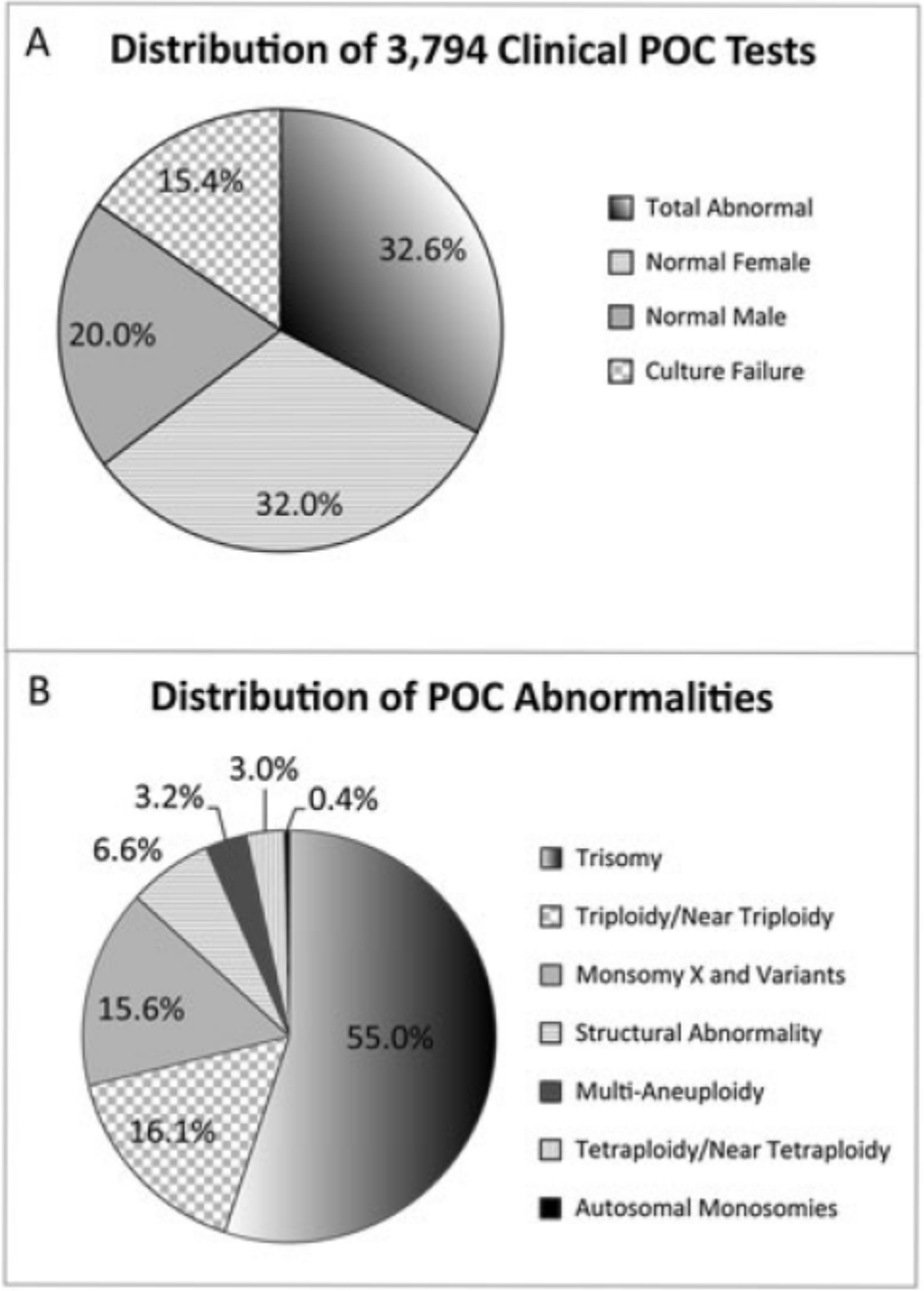
- **Cytogenetic abnormalities are identified in up to 50% of first trimester losses**

Value of genetic testing on pregnancy loss: for patient care

Provides closure for families after a loss

Identifies abnormalities associated with a risk for recurrence

Identification of a genetic abnormality prevents additional costly workup for infertility



ARUP's Laboratory Test Directory

Chromosome Analysis, Products of Conception : 2002288

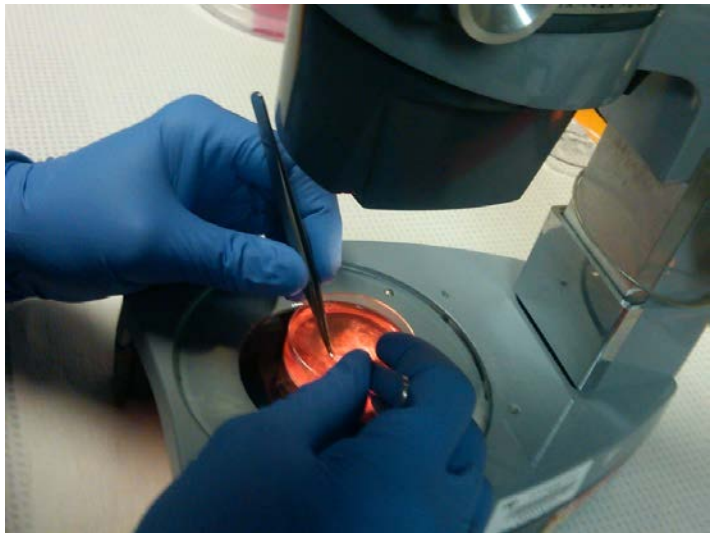
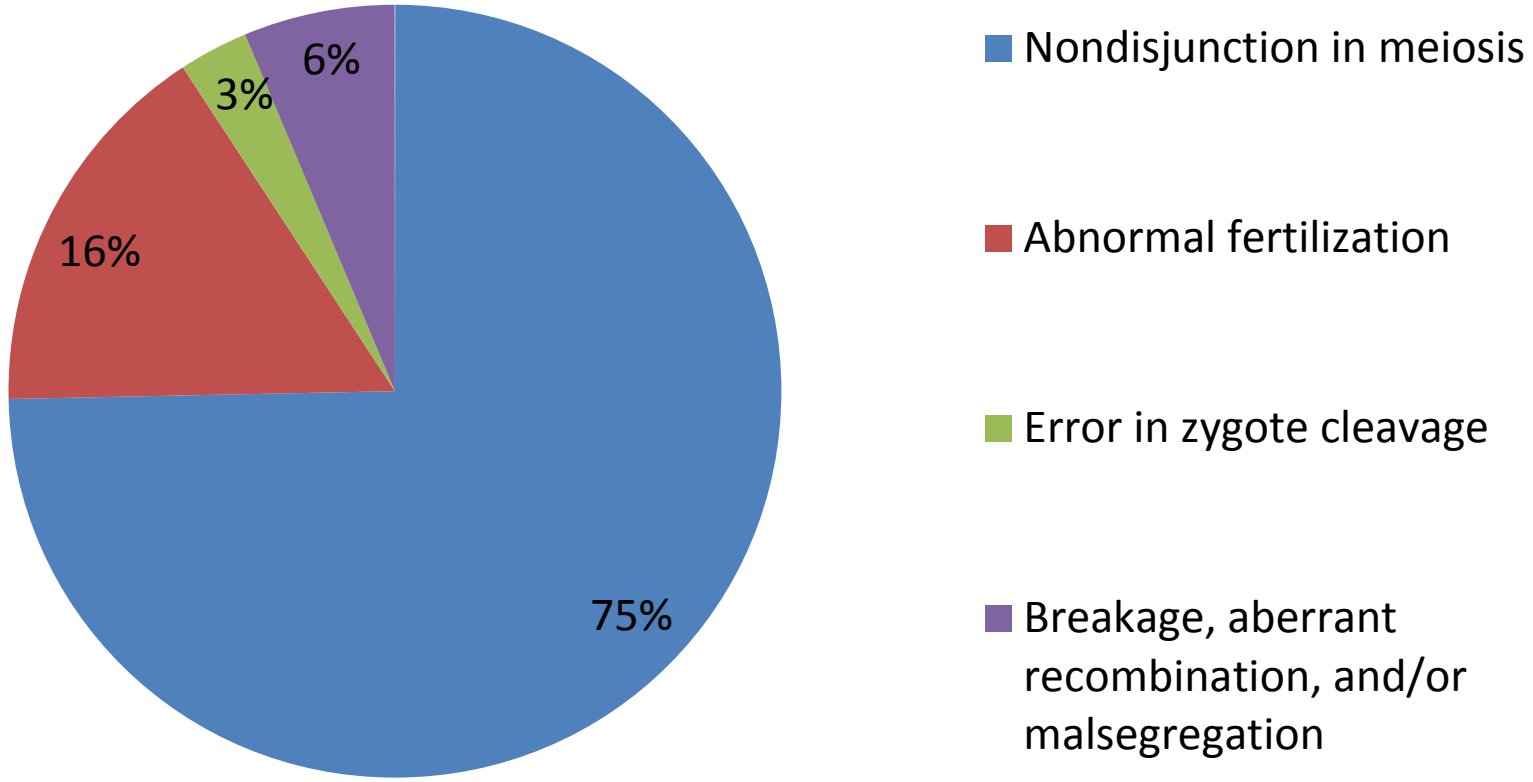


Fig. from Paxton CN et al, *Prenatal Diagnosis* 32, 1–7 [Epub 8 NOV 2012]

Distribution of abnormalities by mechanism



Cytogenetic Findings in POCs

- Aneusomy for virtually every chromosome
- 2-3% of trisomies associated with a potentially heritable translocation
- Secondary aneusomy in polyploidy
  - Triploidy: 9% have secondary trisomy or monosomy
  - Tetraploidy: 14% have secondary trisomy or monosomy
- Mosaicism (17% among all abnormal karyotypes)
  - Eliminating cases of likely maternal cell contamination, the true rate of mosaicism is probably 6%

Anomaly	POCs at ARUP*	Spontaneous abortions**	Live births**
Trisomy 13	1.56%	1.10%	0.01%
Trisomy 18	1.19%	0.84%	0.02%
Trisomy 21	2.86%	2.00%	0.11%
Trisomy 16	3.04%	5.58%	0.00%
Other trisomies	9.52%	11.81%	0.00%
Monosomy X	5.47%	8.35%	0.01%
Sex chromosome trisomies	0.07%	0.33%	0.15%
Triploids	5.50%	5.79%	0.00%
Tetraploids	0.98%	2.39%	0.00%
# Karyotyped	2,763*	3,353**	31,521**

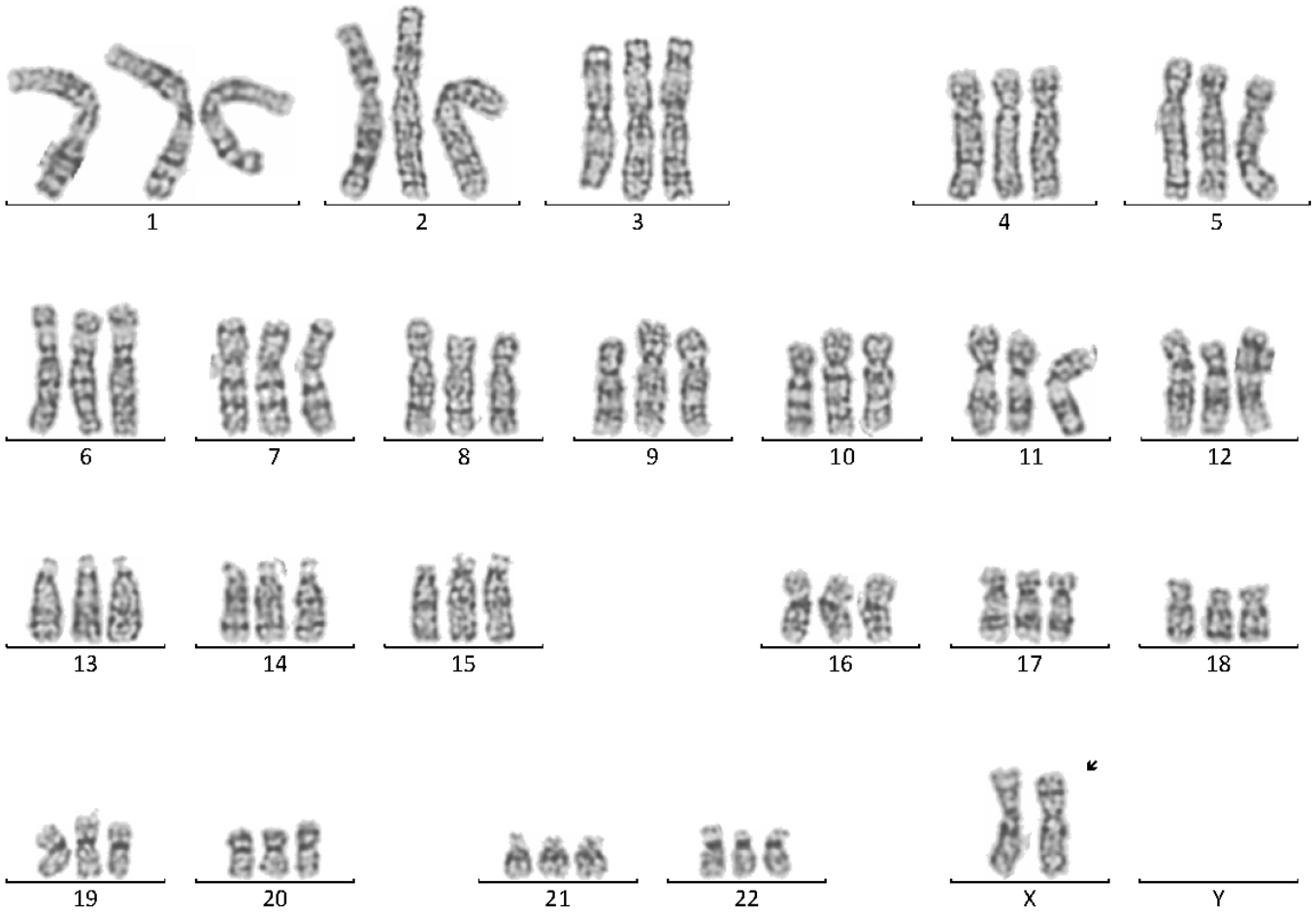
*ARUP data estimated over 32 months of data

**data from Kline, J. and Stein, Z. (1986) The epidemiology of spontaneous abortion. In *Early Pregnancy Failure (Huisjes, H.J.*and Lind, T. eds.), Churchill Livingstone, New York, pp. 240–256.

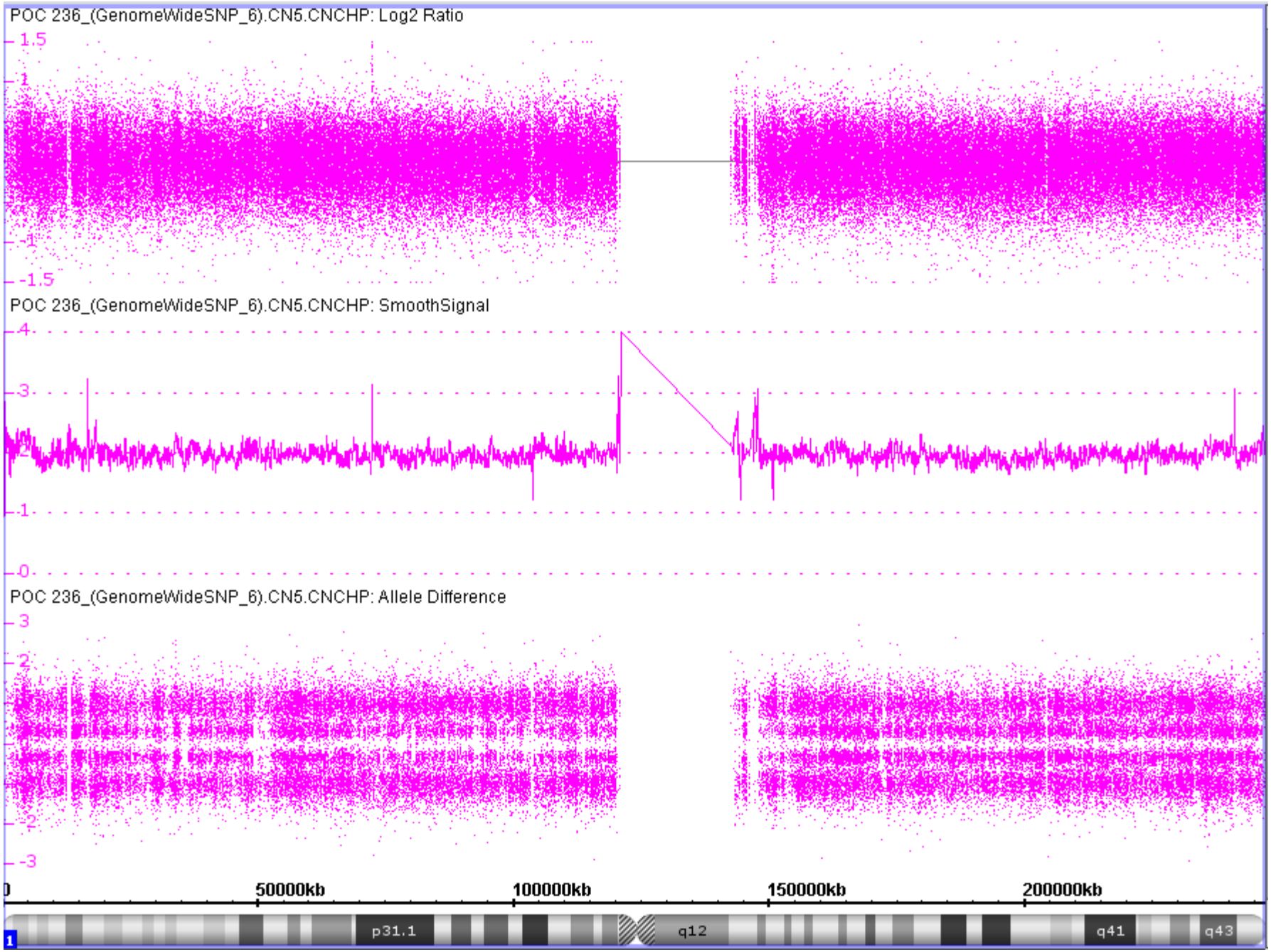
When Is a Karyotype Insufficient?

- Suspected molar pregnancy
- Nonviable tissue
  - Culture failure rate for fresh tissue: ~14%
  - Only paraffin embedded tissue available
- Uncharacterized structural abnormality
  - Marker chromosome
  - Additional material of unknown origin
  - Submicroscopic copy number variants (CNVs)

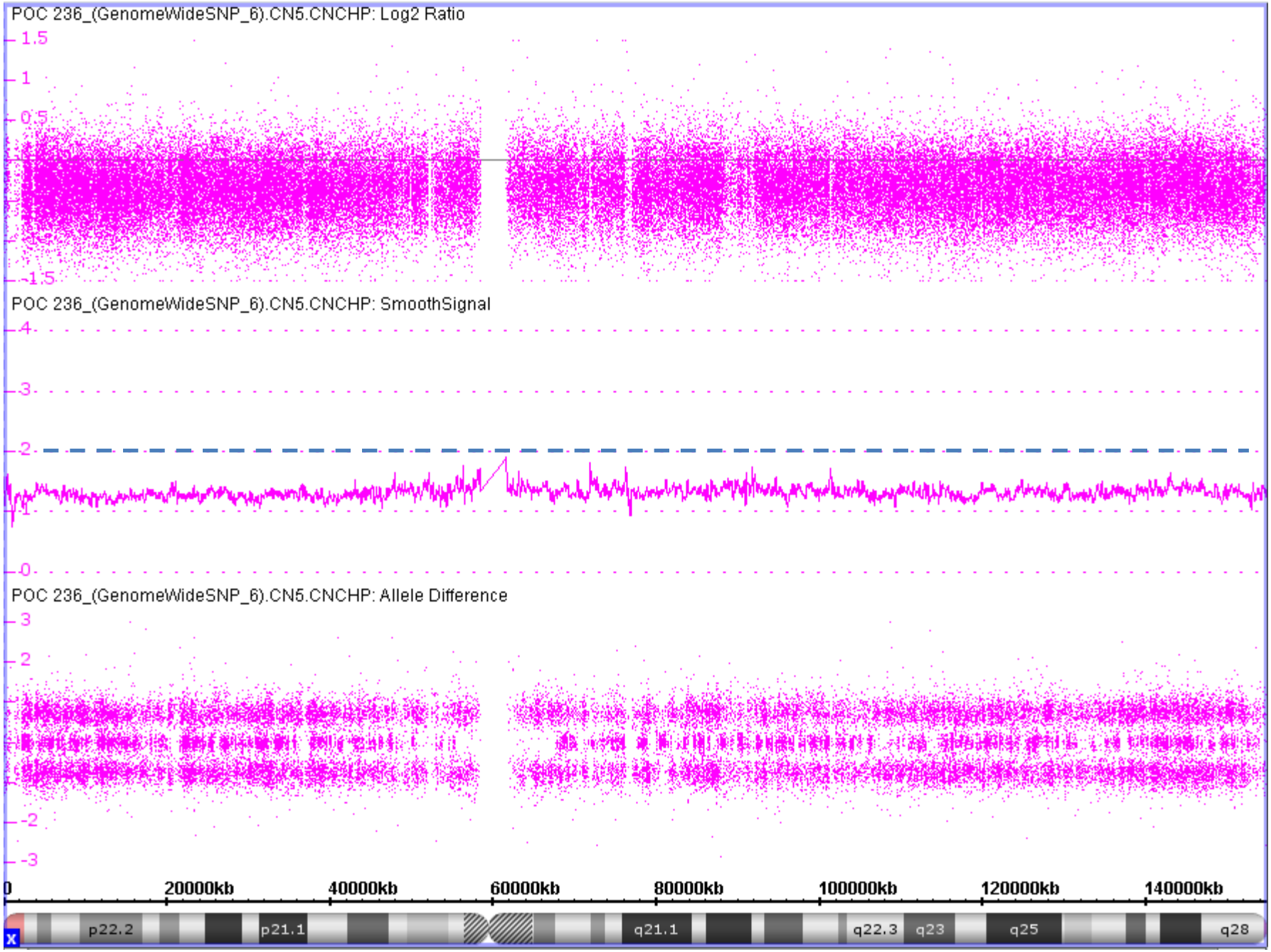
68,XX



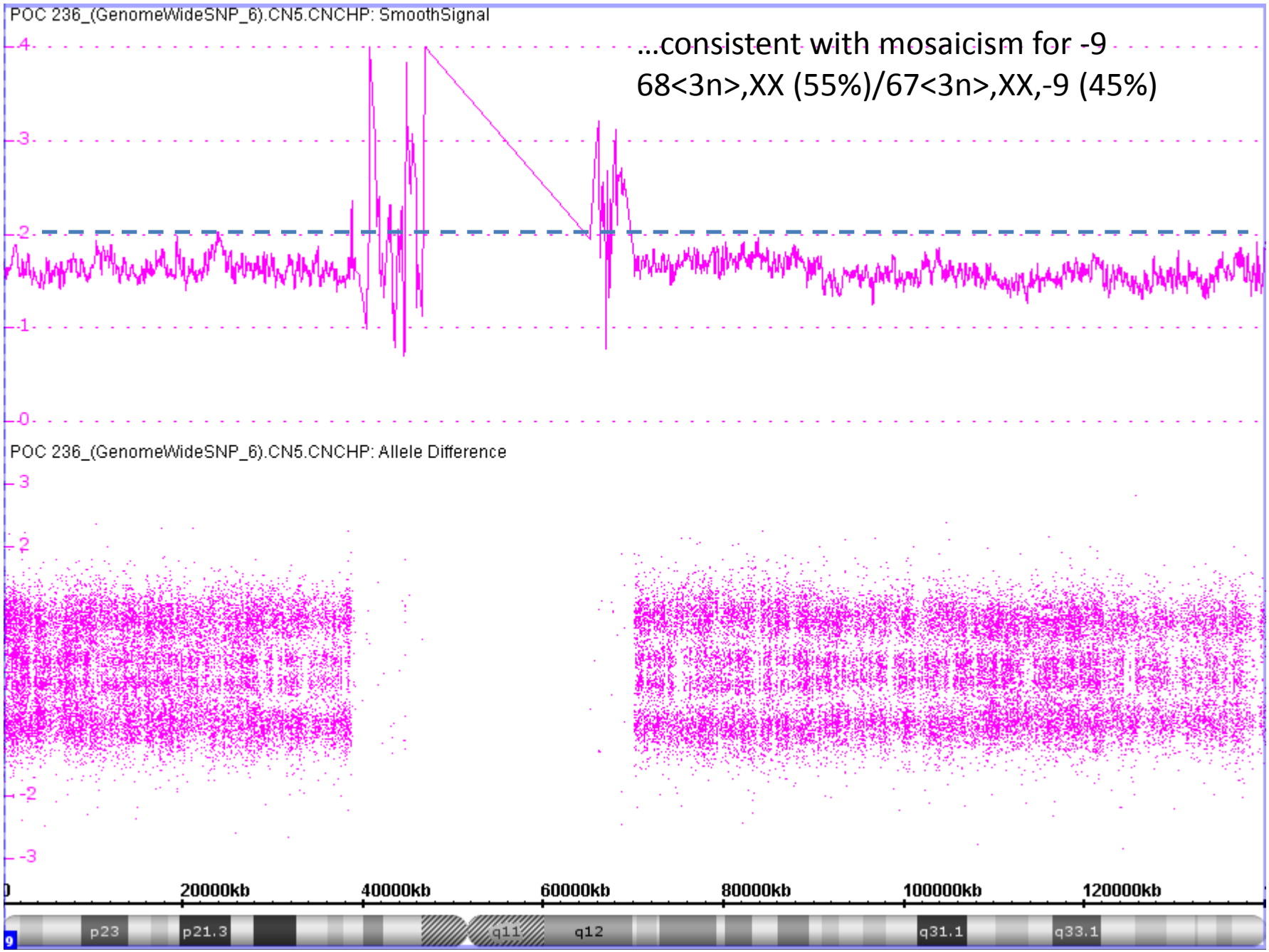
Chromosome 1-22: 4 allele tracks, copy number 2, consistent with triploidy



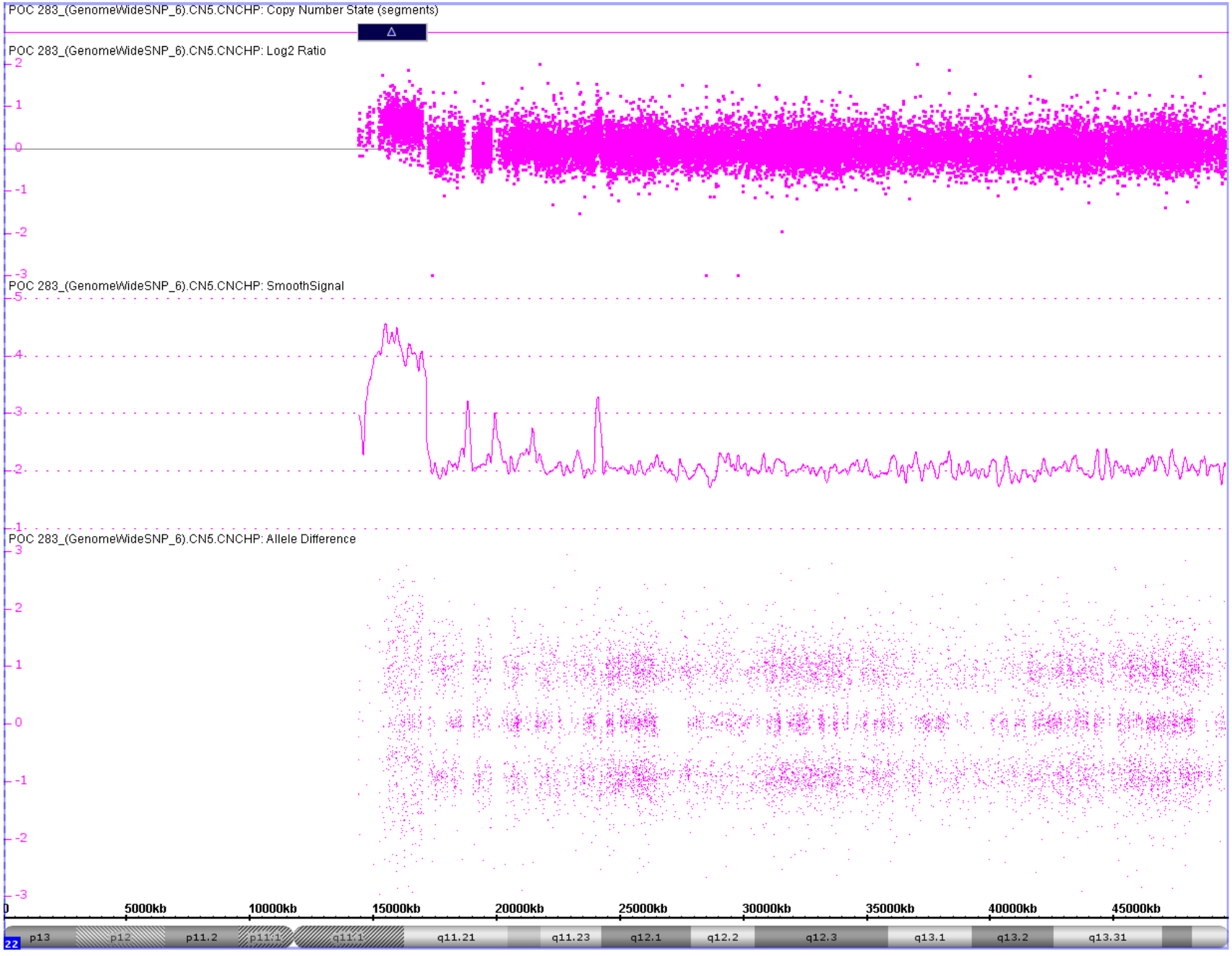
X chromosome shows 3 tracks, as for 46,XX, but copy number approx. 1.3



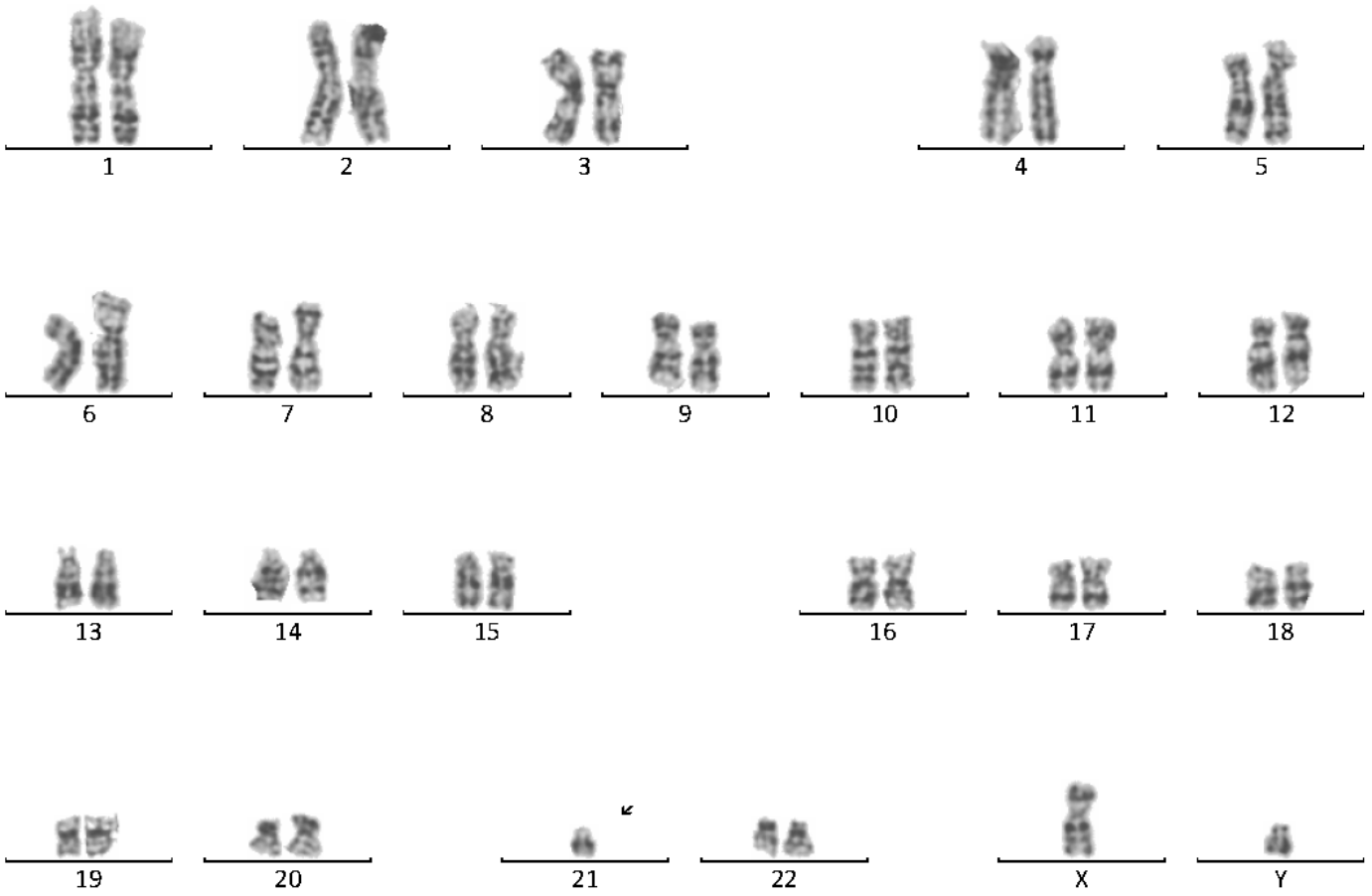
Chromosome 9 shows copy number approx. 1.7



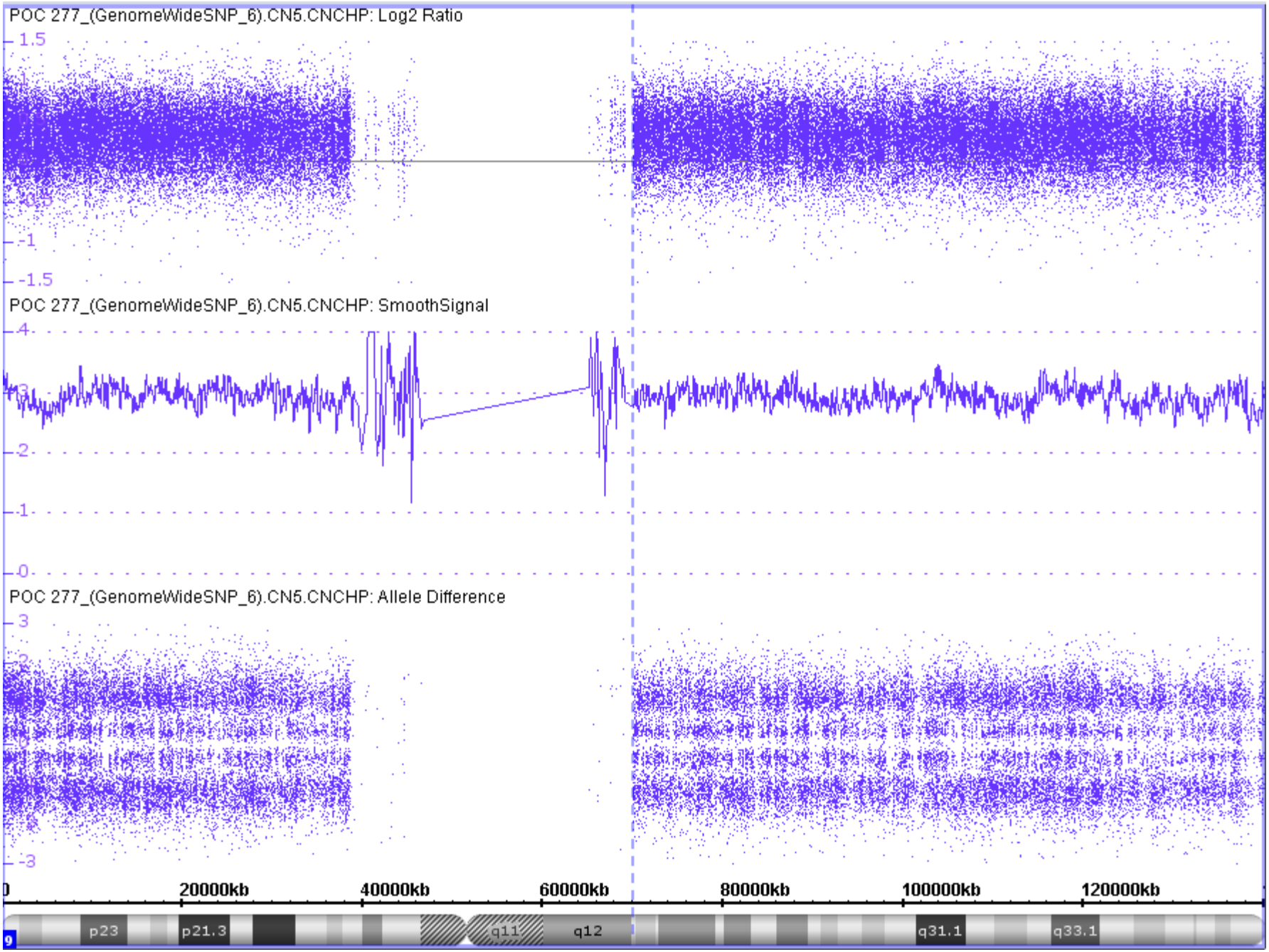
Characterization of a marker chromosome



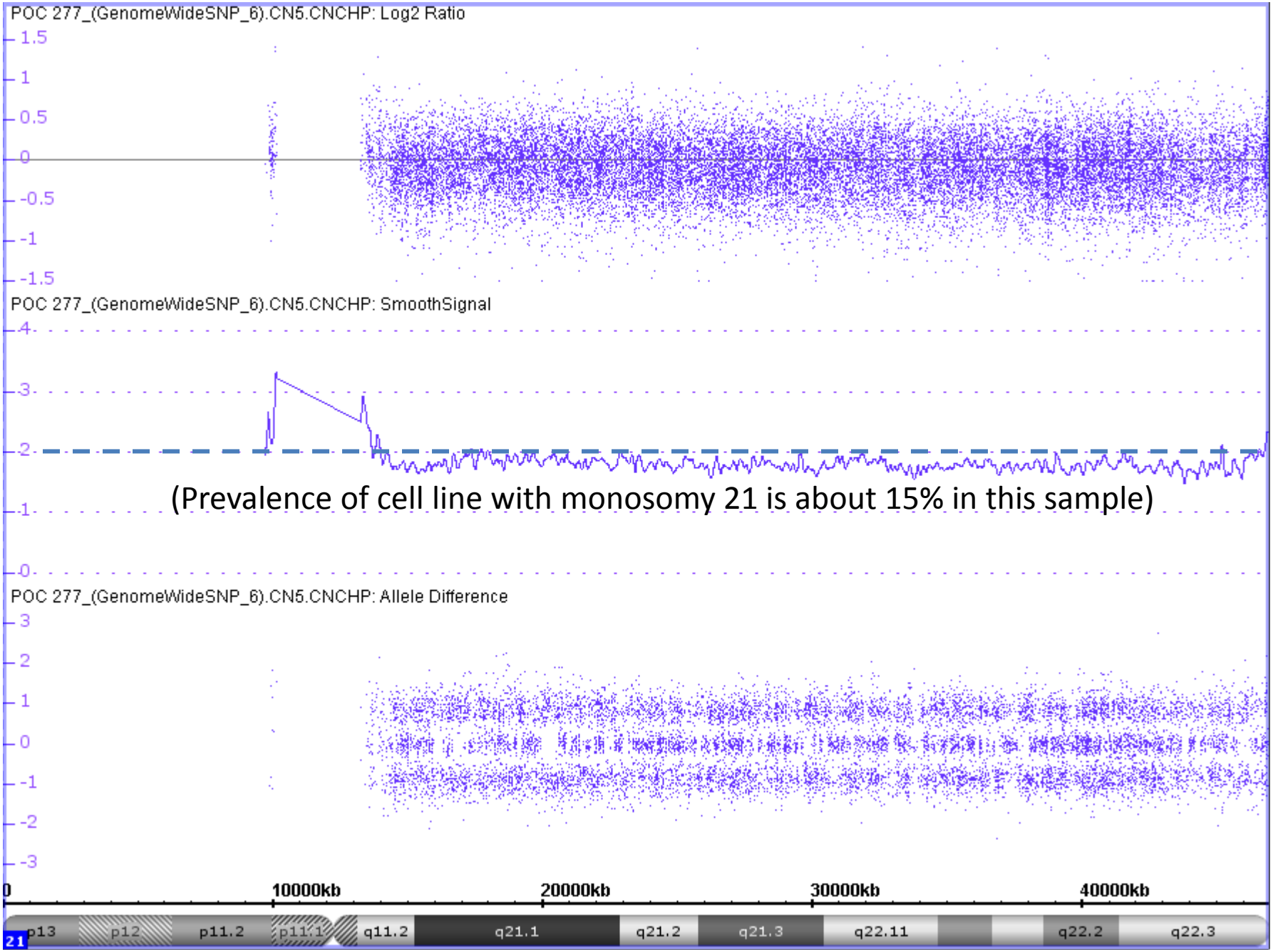
45,XY,-21[8]/46,XX[11]



Chromosome 9 gain (copy number = 3)

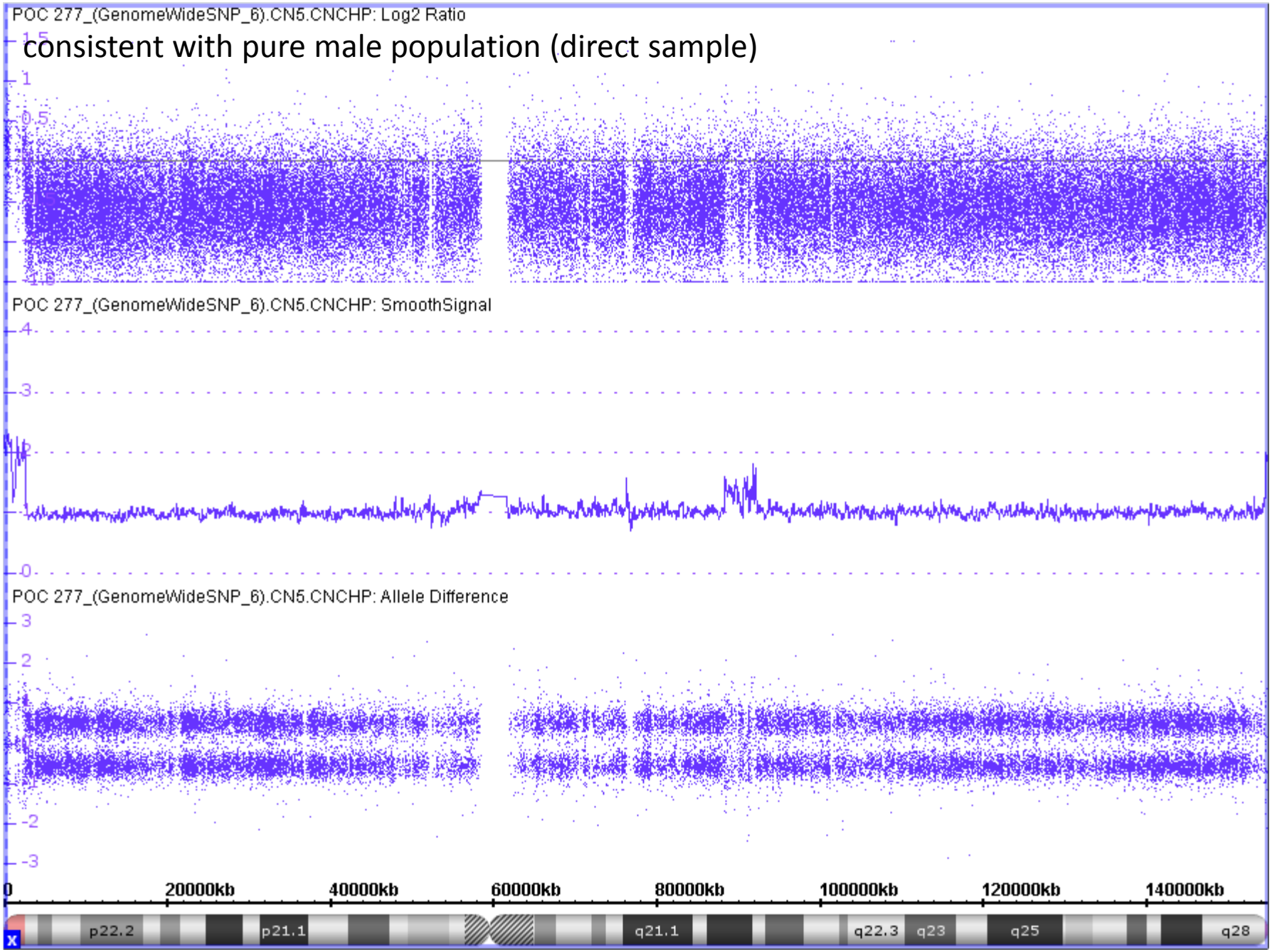


Chromosome 21 mosaic loss (copy number approx. 1.85)



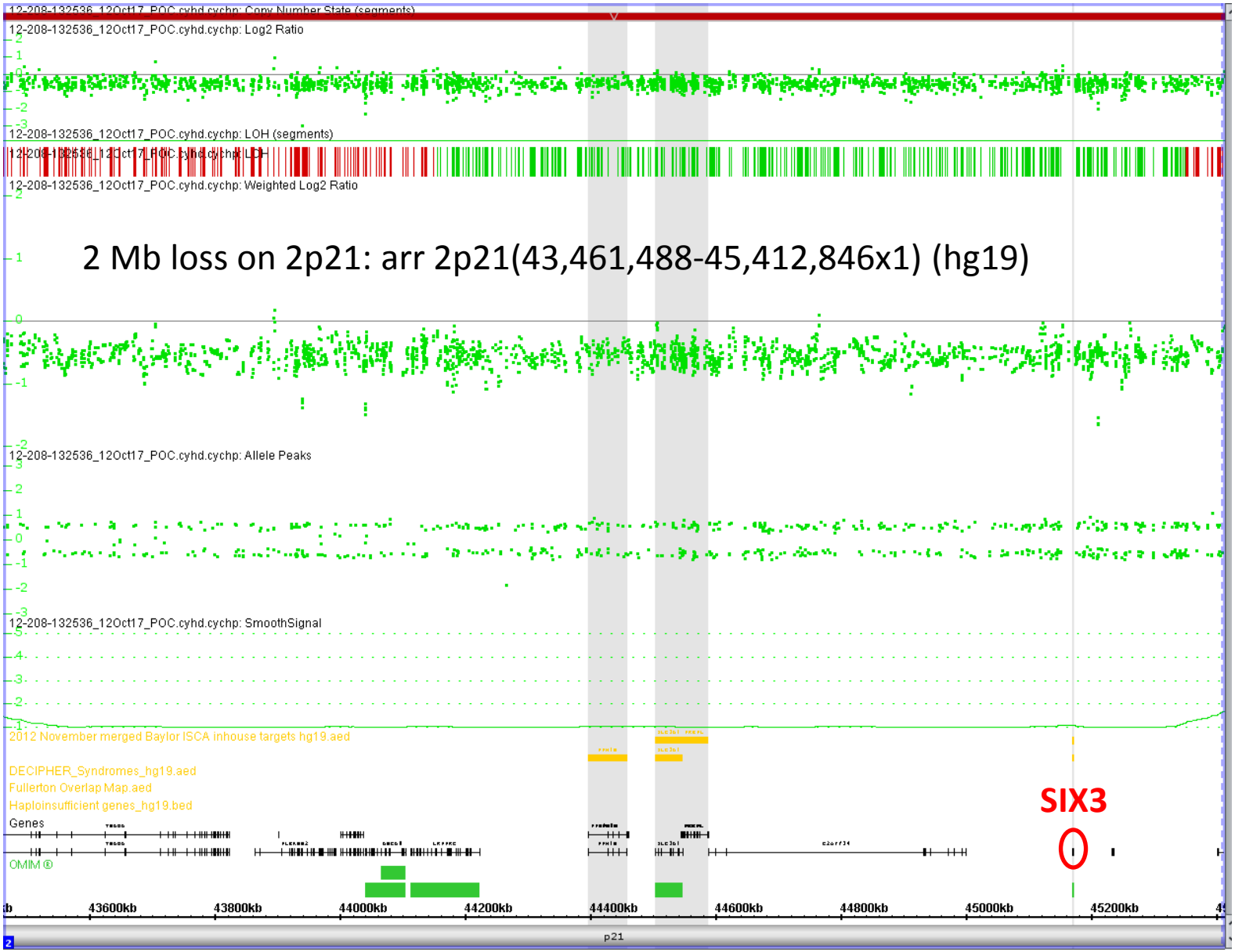
(Prevalence of cell line with monosomy 21 is about 15% in this sample)

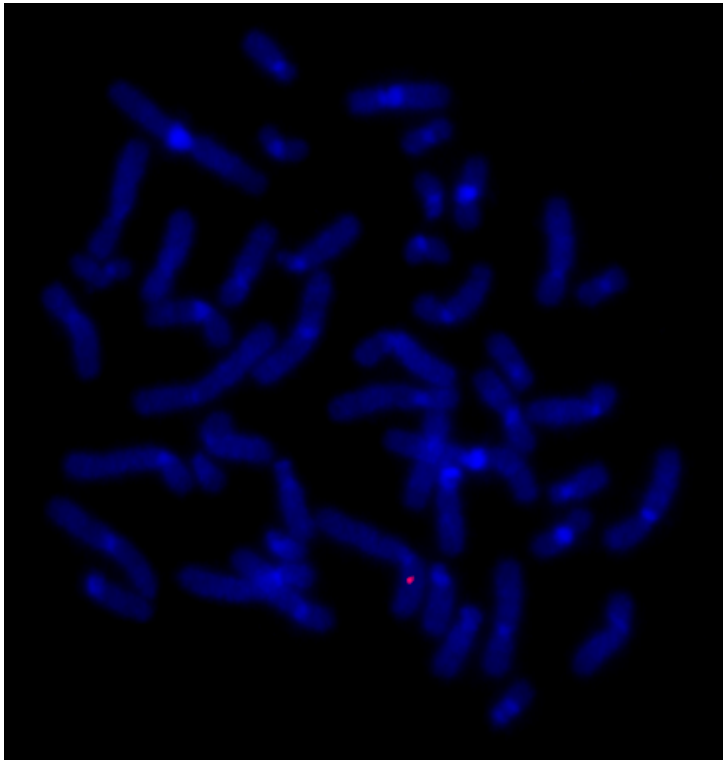
Chromosome X shows copy number = 1, Y copy number = 1 (not shown),



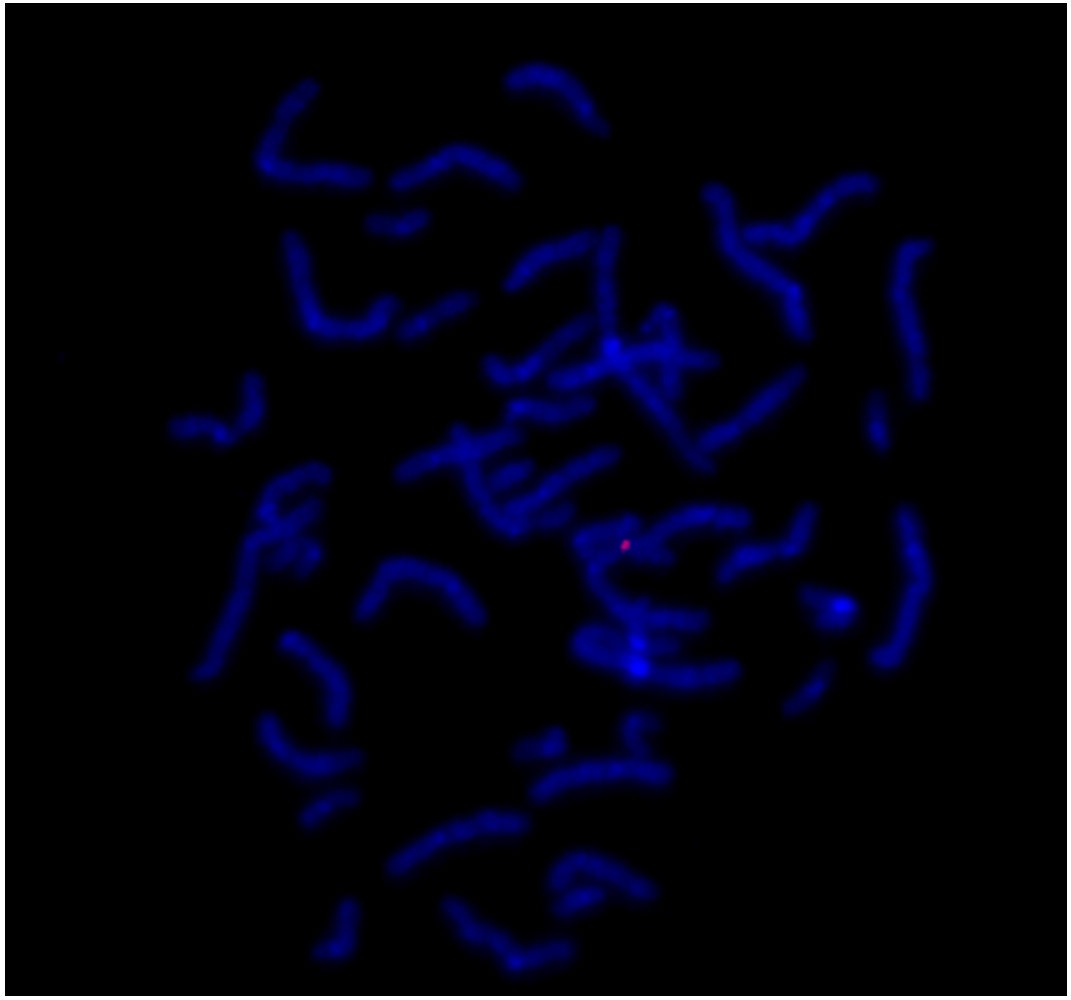
consistent with pure male population (direct sample)

Fetal demise – alobar holoprosencephaly



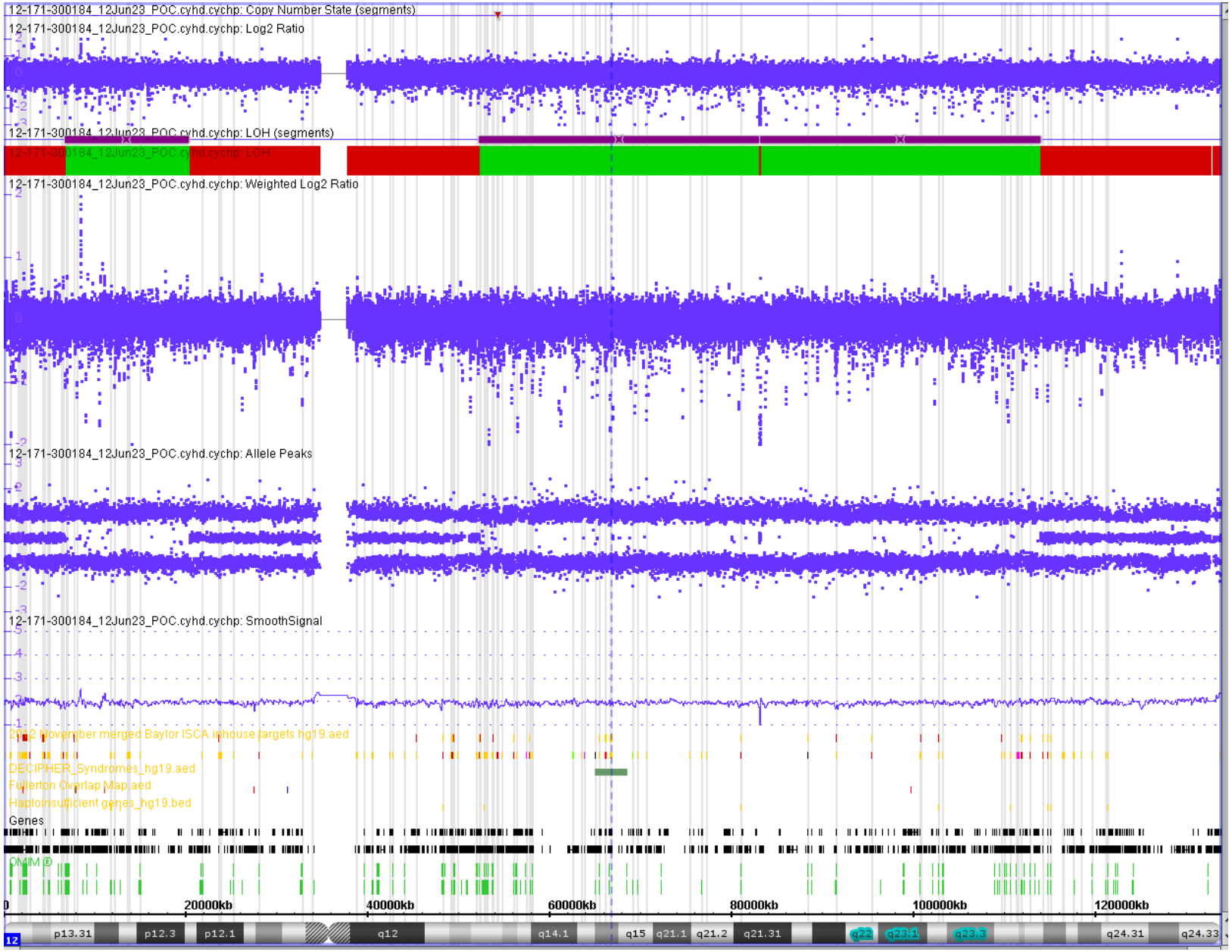


Metaphase FISH on
POC material
(used as positive control)
RP11-489K22 BAC (Empire Genomics)



Mother carries same deletion by FISH

Absence of heterozygosity affecting 22.5% of genome



Stretches of homozygosity

- May indicate:
  - Uniparental disomy if present on a single chromosome
  - Identity by descent if present in numerous independent regions of the genome
  - Complete hydatidiform mole if 100% homozygous (monospermy) or ~50% homozygous (dispermy)
- Associated with increased risk for autosomal recessive condition
  - In pregnancy loss / fetal demise, consider potentially lethal AR condition

Microarray for pregnancy loss: summary

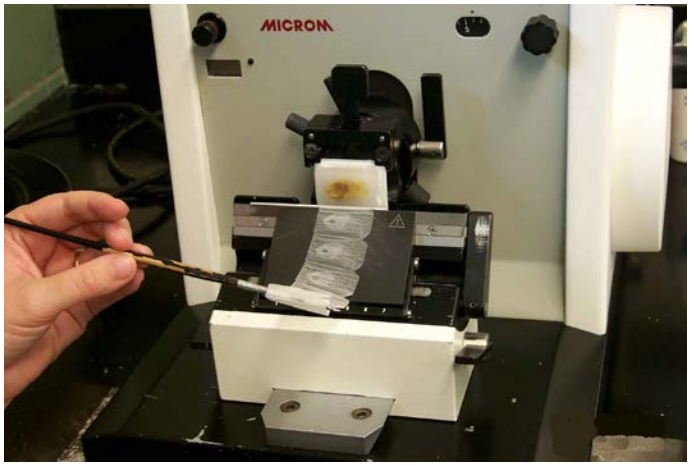
- Chromosomal microarray can be successfully applied to pregnancy loss samples, may yield different information than that provided by the karyotype, and is more sensitive and specific for characterizing clinically relevant genomic imbalances
- Karyotype remains the most versatile method for detecting mosaicism and some polyploidy (tetraploidy); microarray is most valuable as a reflex test for POCs with a normal karyotype or POCs which fail to grow in culture

3. MOLAR PREGNANCY

Hydatidiform Mole

- Associated risk for persistent trophoblastic disease
- Complete mole is often detected clinically, whereas partial mole is more often detected by the pathologist
  - BUT: histologic diagnosis is unreliable
- 3 major ancillary diagnostic tests (FFPE):
  - p57 immunohistochemistry (complete mole)
  - Flow cytometry (partial mole)
  - Microsatellite genotyping (complete and partial mole)

ARUP LABORATORIES | National Reference Laboratory



ARUP's Laboratory Test Directory

Molar Pregnancy, 16 DNA Markers : 0051755

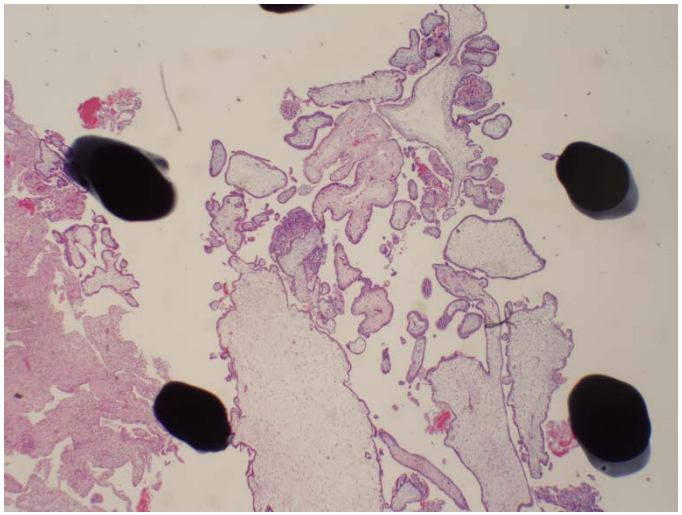
Mnemonic: MOL PREG

Ordering Recommendation:	Diagnose complete or partial molar pregnancy.
Methodology:	Polymerase Chain Reaction/Fragment Analysis
Performed:	Mon-Fri
Reported:	Within 14 days

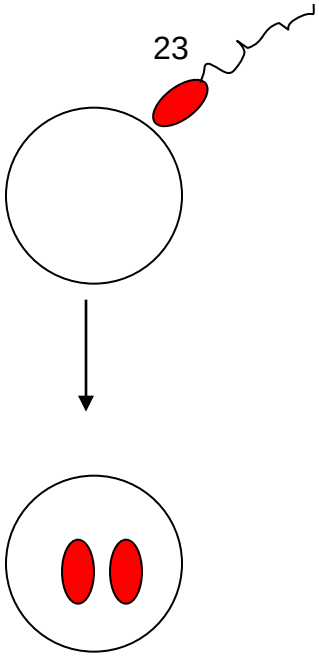
Collect: Products of conception.

Specimen Preparation: Formalin fix and paraffin embed tissue containing areas of villi and of decidua. In some cases, decidua may not be present in sufficient amounts, and a maternal blood sample may be requested. Transport tissue block.

Storage/Transport Temperature: Room temperature. Ship in a cooled container during the summer months.

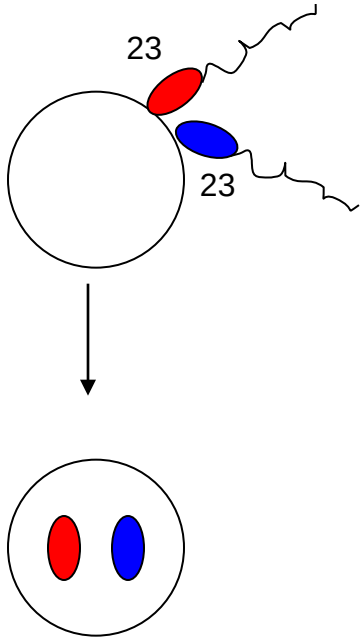


Complete hydatidiform mole



46,XX

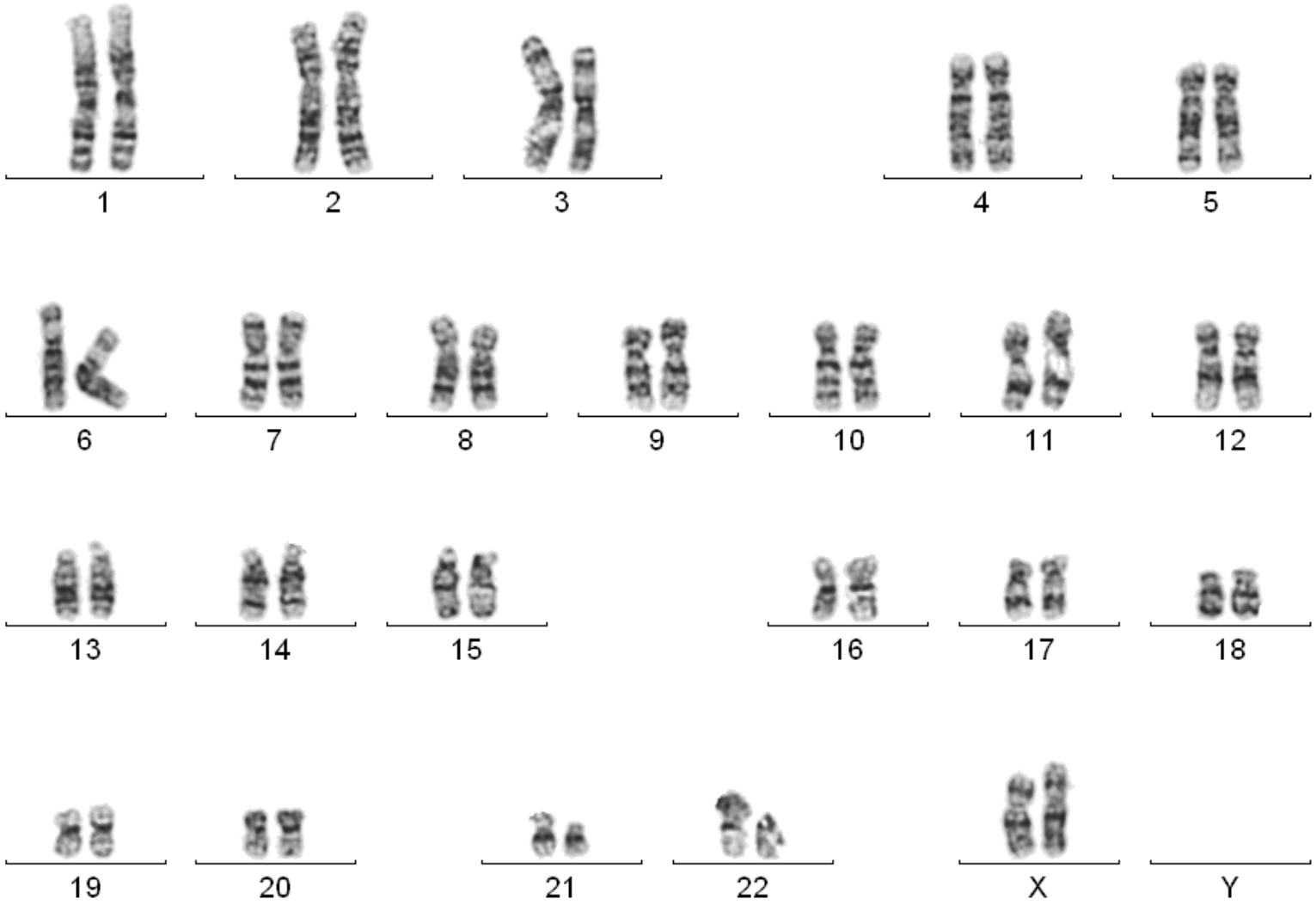
Monospermy with endoreduplication



46,XX or 46,XY

Dispermy

- Diploid
- Complete uniparental disomy across entire genome
- No maternal DNA
- 15% risk for persistent gestational trophoblastic disease



46,XX

5 STR loci: comparison of genotypes of villi (“fetus”) and decidua

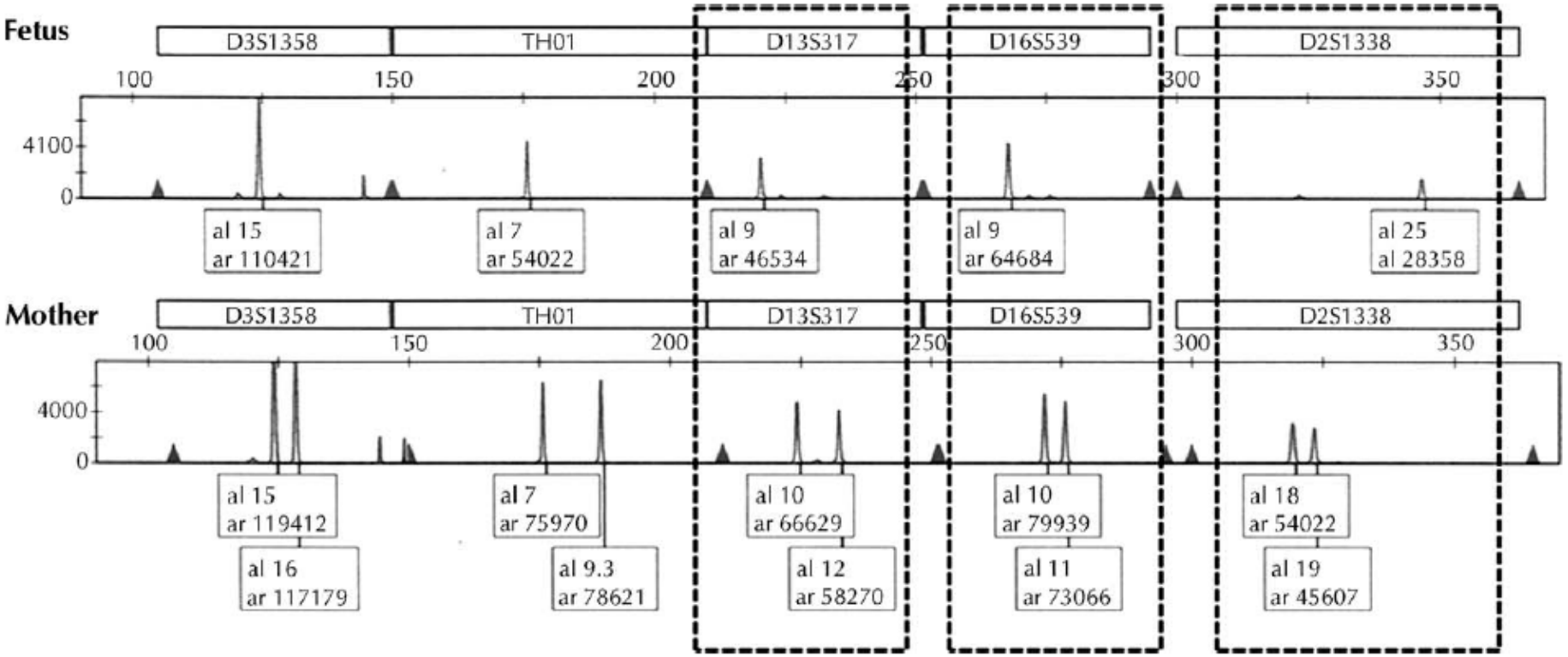
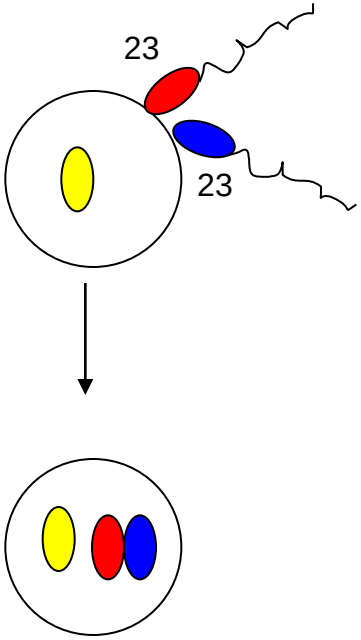
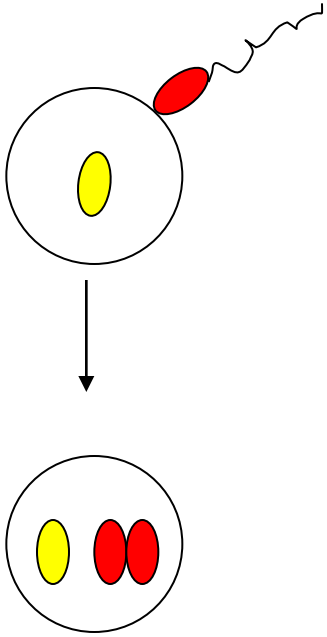


Fig. 1 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. *Archives of Pathology and Laboratory Medicine* [In Press]

Partial hydatidiform mole

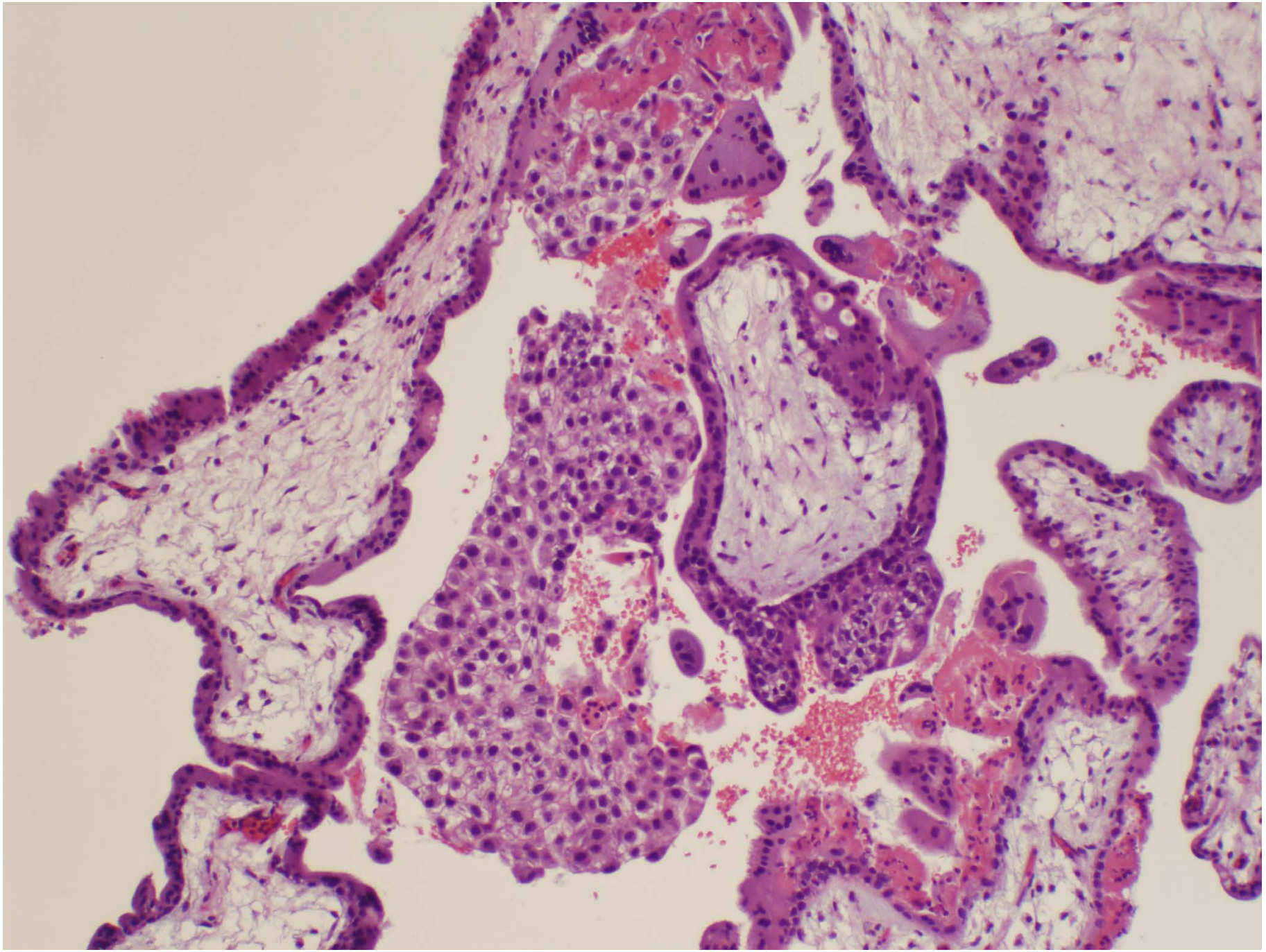


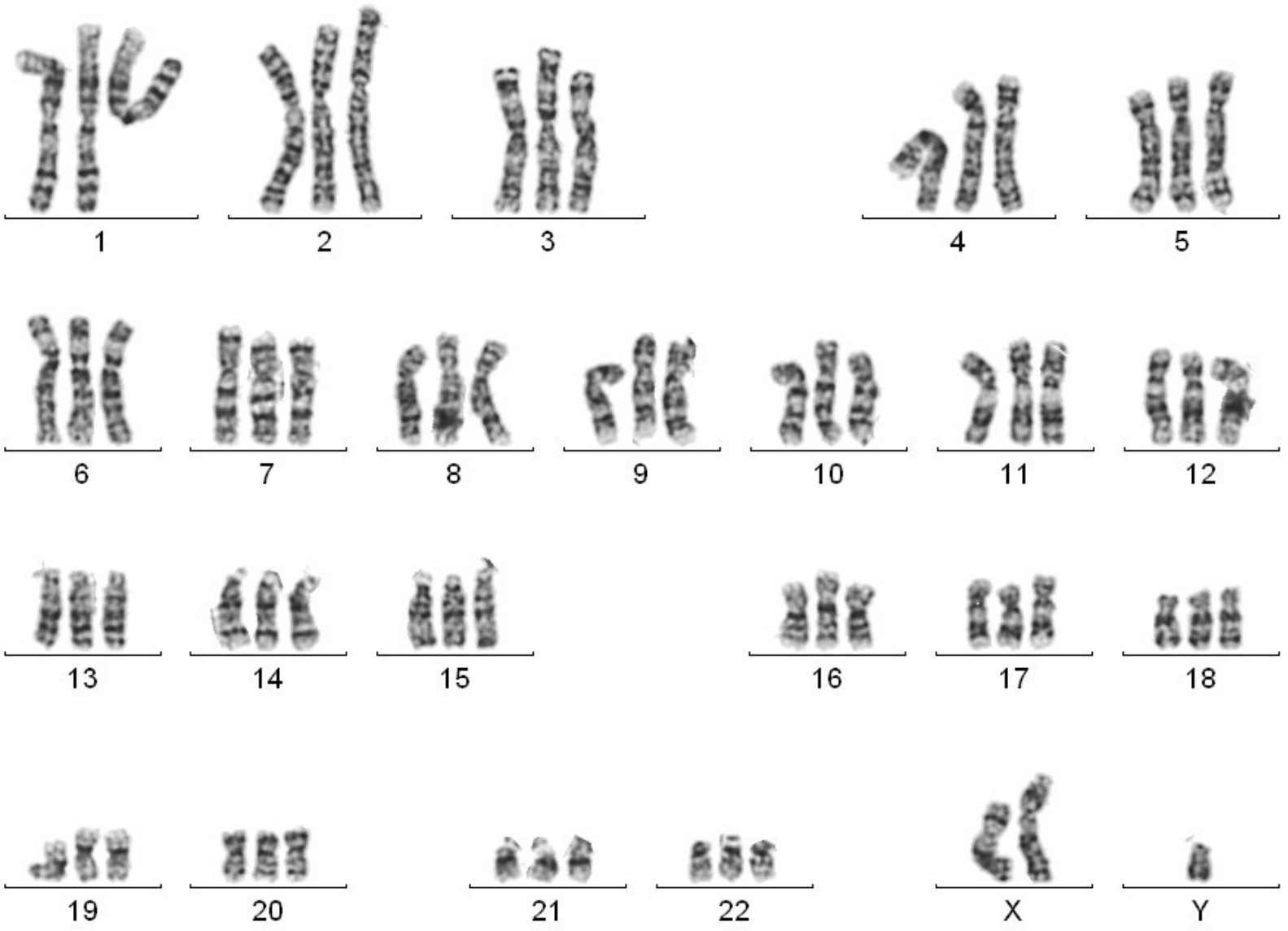
dispermy



monospermy with endoreduplication
(theoretical mechanism)

- Triploid
- 2:1 paternal: maternal DNA contribution
- 0.5% risk for persistent gestational trophoblastic disease
    - not a risk factor for choriocarcinoma?





69,XXY

4 STR loci: comparison of genotypes of villi (“fetus”) and decidua

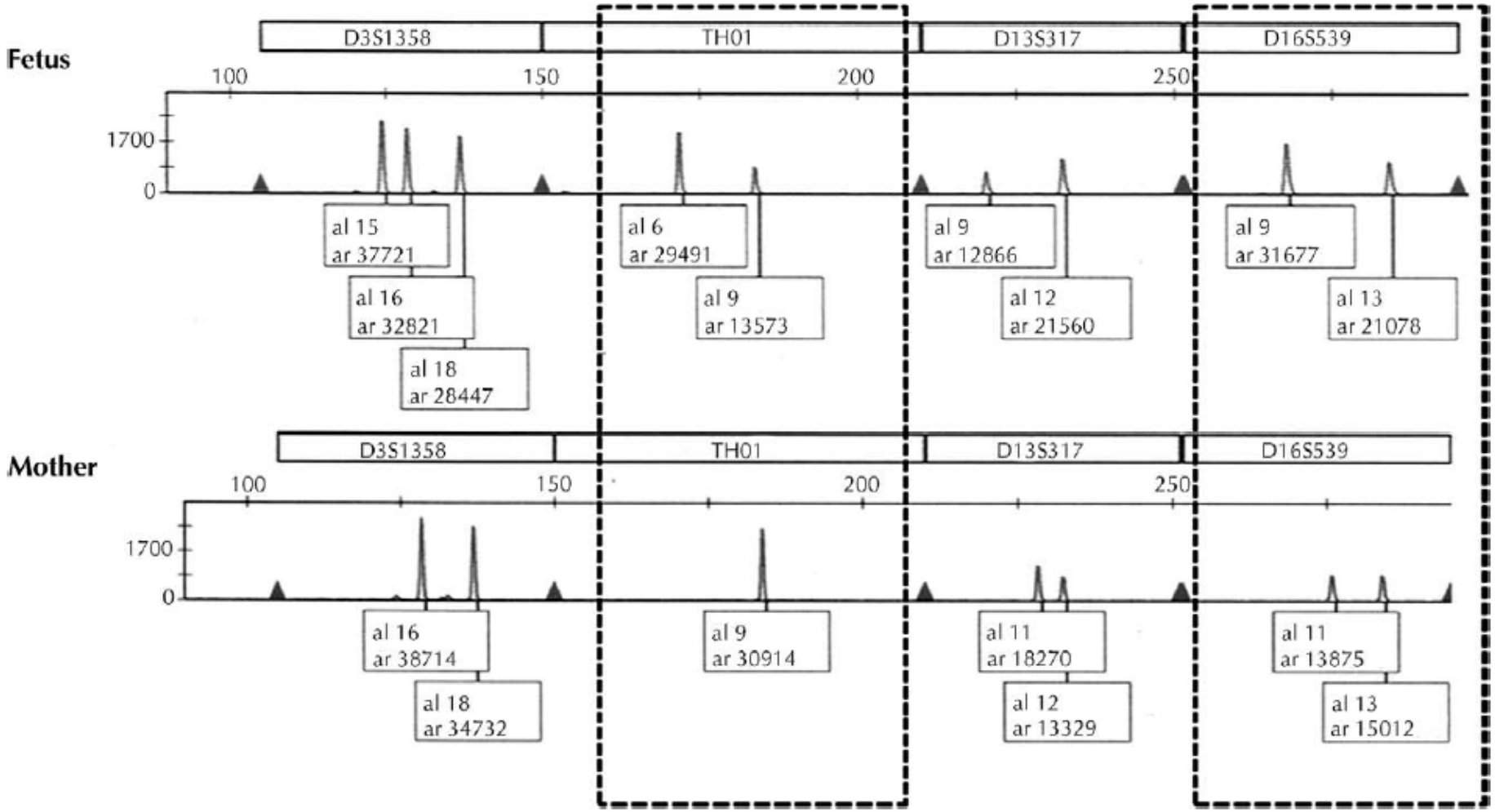
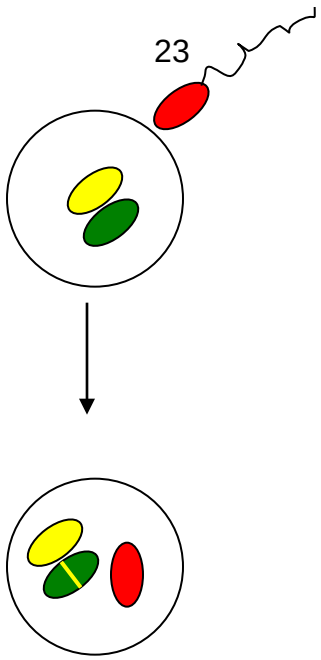
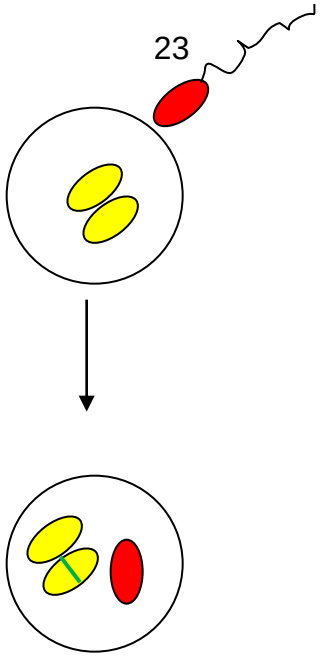


Fig. 2 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. *Archives of Pathology and Laboratory Medicine* [In Press]

Maternally derived triploidy (digyny): diploid egg



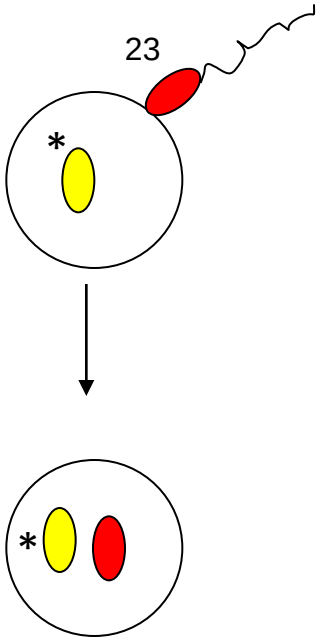


69,XXX or 69,XXY

69,XXX or 69,XXY

- Triploid
- 1:2 paternal: maternal DNA contribution
- Fetal anomalies
- NO risk for persistent gestational trophoblastic disease

Nonmolar hydropic abortion (HA)

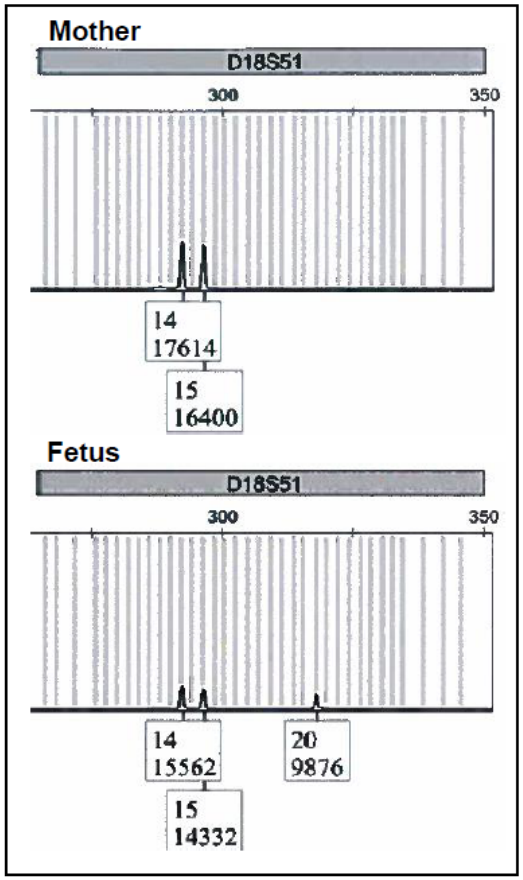


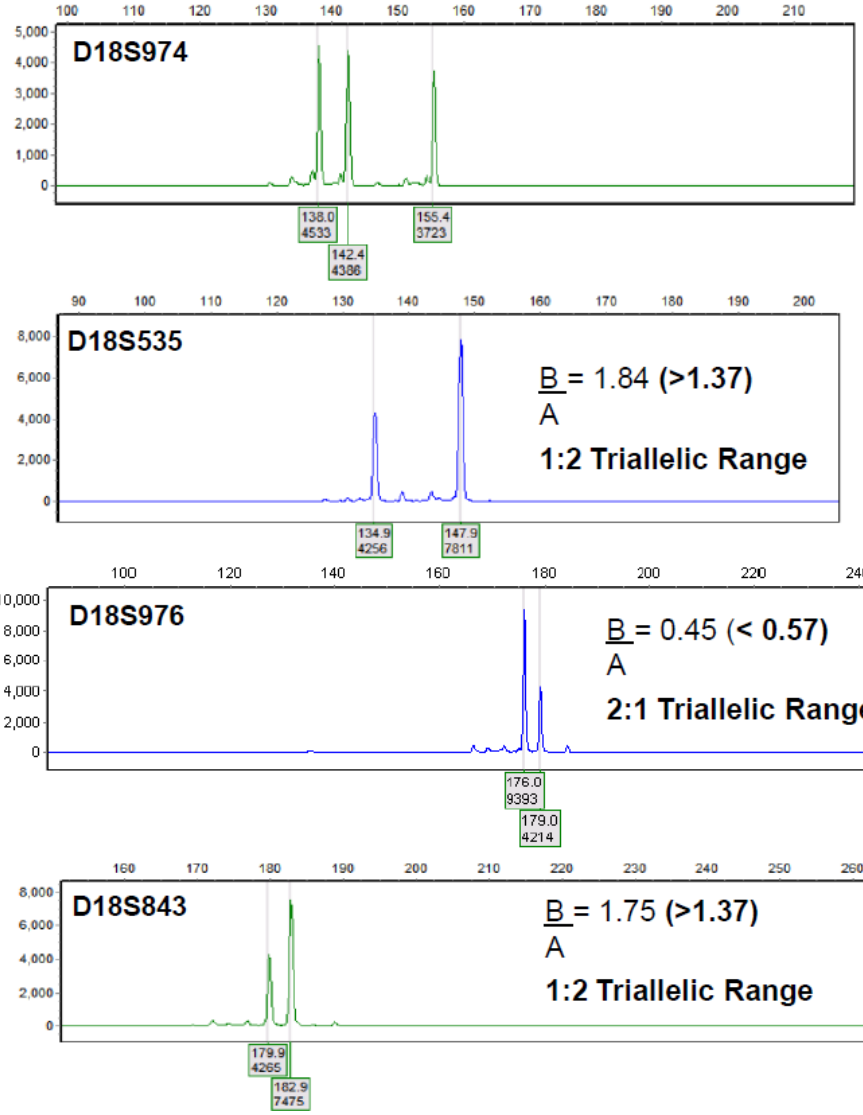
Autosomal trisomy, sex chromosome aneuploidy,
Mendelian disorders, non-genetic causes

May simulate molar pregnancy by histopathology

- Trophoblastic proliferation
  - particularly +7, +15, +21, or +22
  - trophoblastic hypoplasia may also be seen (e.g. trisomy 18)
- Villous edema / hydrops

Incidental Trisomy Detection
(10/54 non-molar cases; not reported)





Unusual Case of Chimerism (Androgenetic / Biparental)

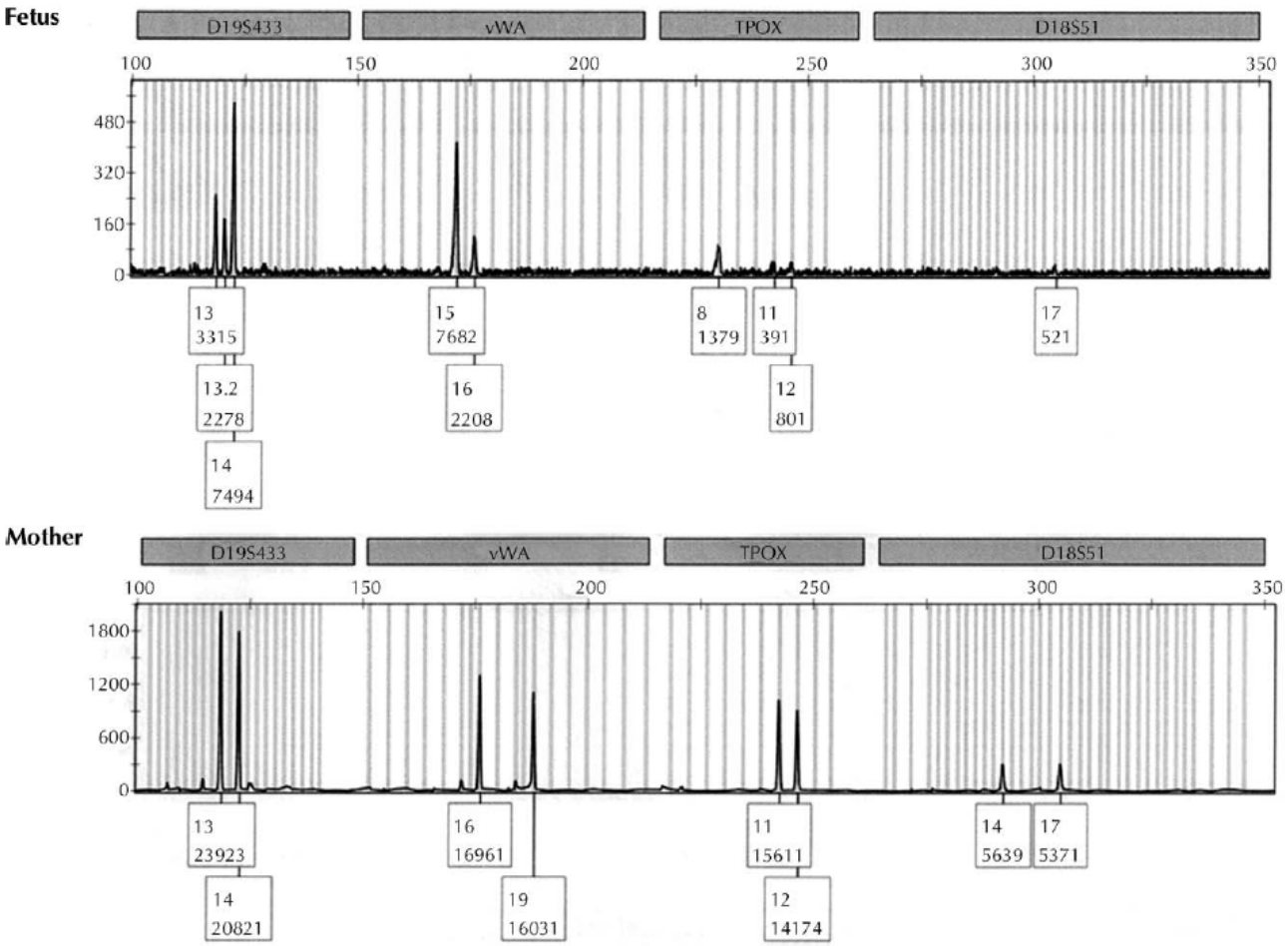
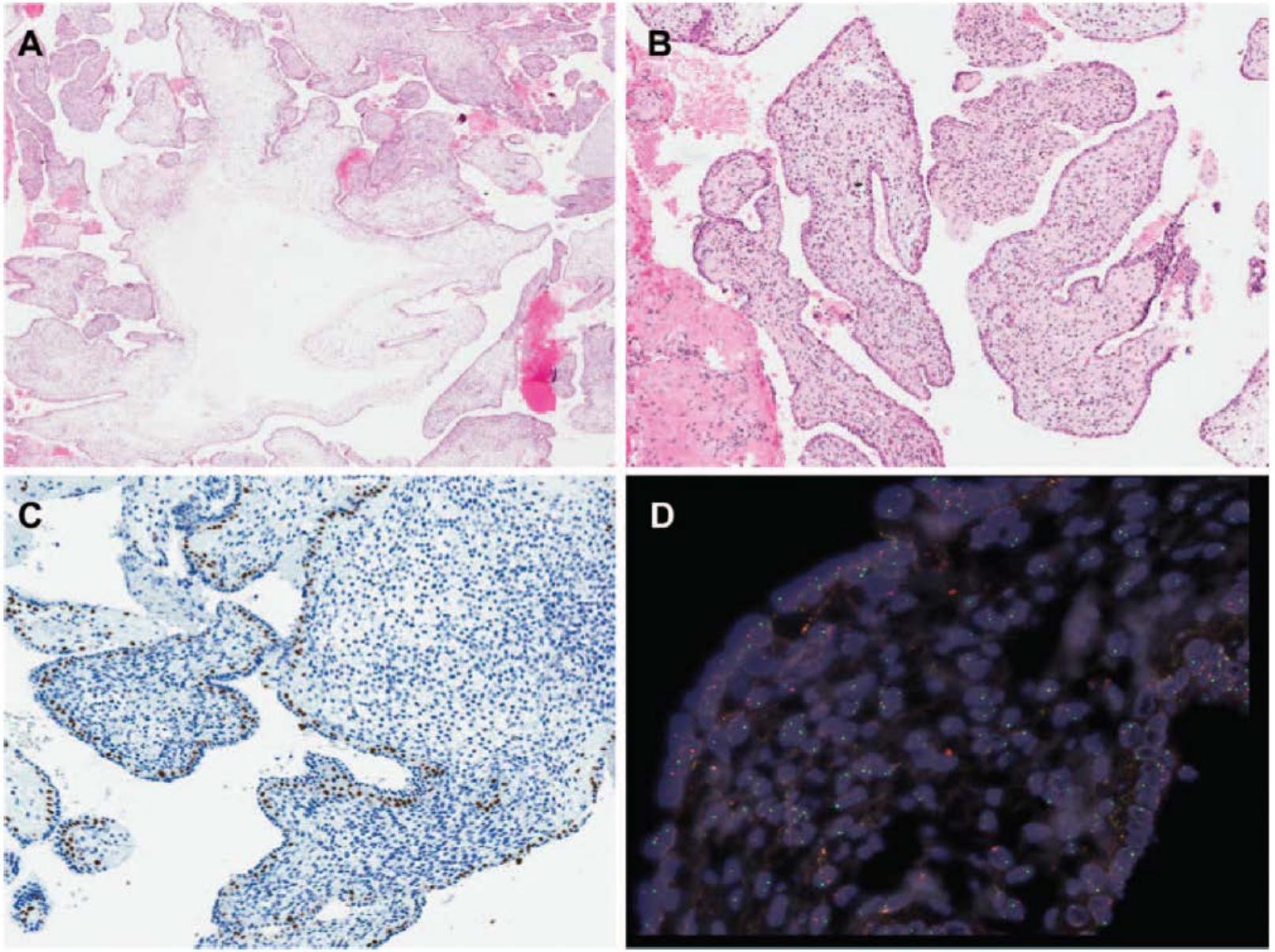


Fig. 4 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. *Archives of Pathology and Laboratory Medicine* [In Press]



p57 immunohistochemistry

FISH with X and Y centromere probes

Fig. 5 from Furtado et al, Diagnostic Utility of Microsatellite Genotyping for Molar Pregnancy Testing. *Archives of Pathology and Laboratory Medicine* [In Press]

Conclusions

- New technology has introduced new options and new testing algorithms for prenatal screening and diagnosis
- Cytogenetic analysis is the most versatile method for whole genome analysis of pregnancy loss samples
  - Microarray is a useful adjunct method, largely because it can yield results in samples that fail to grow in culture
- Accurate diagnosis of hydatidiform mole relies upon ancillary testing