Prenatal cytogenetic diagnosis : laboratory aspects

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Goal of prenatal diagnosis

To inform couples about the risk of a birth defect or genetic disorder in their pregnancy

To provide them with informed choices on how to manage that risk (genetic counseling)

Principal indications

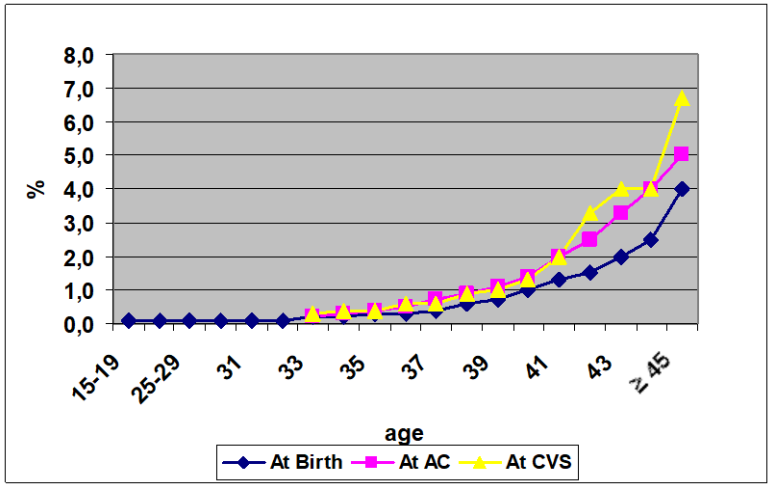
INVASIVE

NON

Known family history -> elevated risk for a specific genetic disorder

Ultrasound abnormalities

Advanced maternal age



Data from Benn PA, Hsu LY, 2003

Invasive testing

Chorionic villus sampling

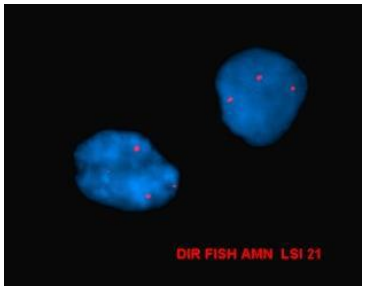
Amniocentesis

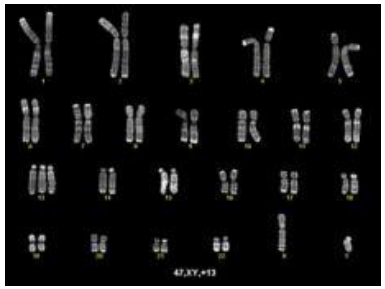
- **Cordocentesis: after 20th week of gestation**
  → **fetal blood**

- **Preimplantation genetic diagnosis**
  → **another presentation**

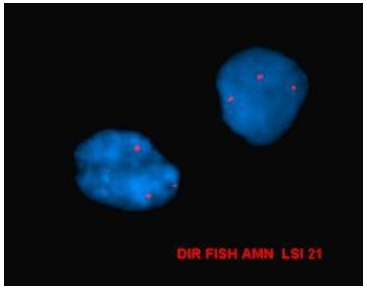
Evolution of prenatal diagnosis

- <2010
  → All invasive samples

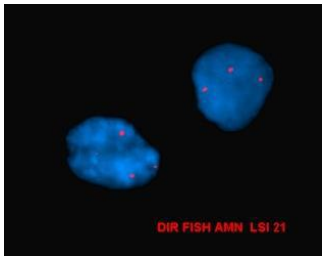




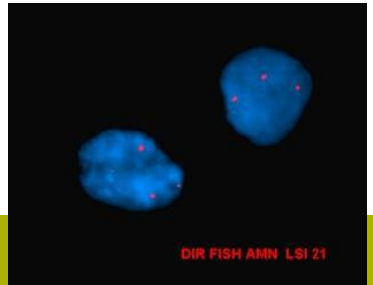
- 2010
  → Ultrasound anomalies



  → Other indications



- 2013
  → All invasive samples

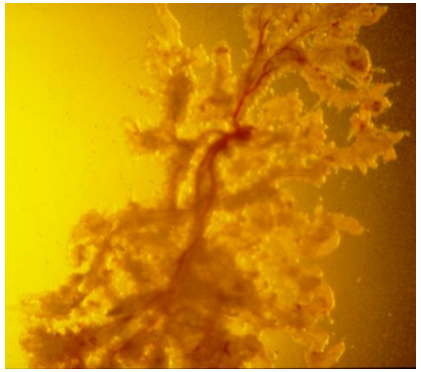


Consensus 8 genetic centers in Belgium

- **From 2013 in Belgium: for all prenatal samples = aCGH**
  - → **Consensus:**
    - **Use 60K arrays (or comparable resolution)**
    - **Always test for maternal cell contamination**
    - **Always obtain a parental blood sample**
    - **Always have at least 1 backup flask in culture**
    - **Testing for triploidy is done (FISH, STR, SNP array)**
    - **A rapid aneuploidy test is not necessary if the TAT is less than one week**
      - **Batching samples -> benefits for cost (lab work)**

Invasive testing

- **Chorionic villus sampling (CVS) :**
  From 11 - 12th week of pregnancy

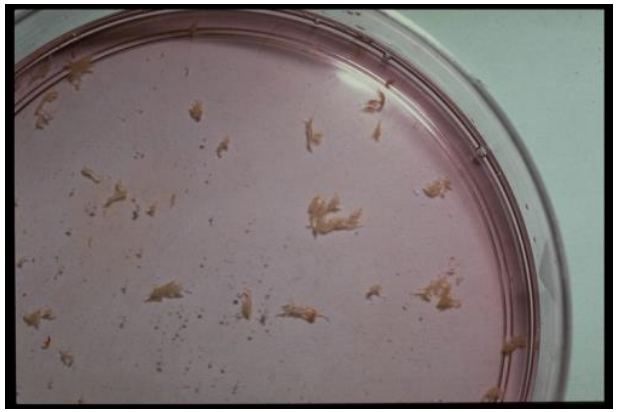


- **Amniocentesis :**
  From 14 - 16th week of pregnancy

⟶ **in our laboratory**

Chorionic villus sampling (CVS)

Prenatal culture room-CVS

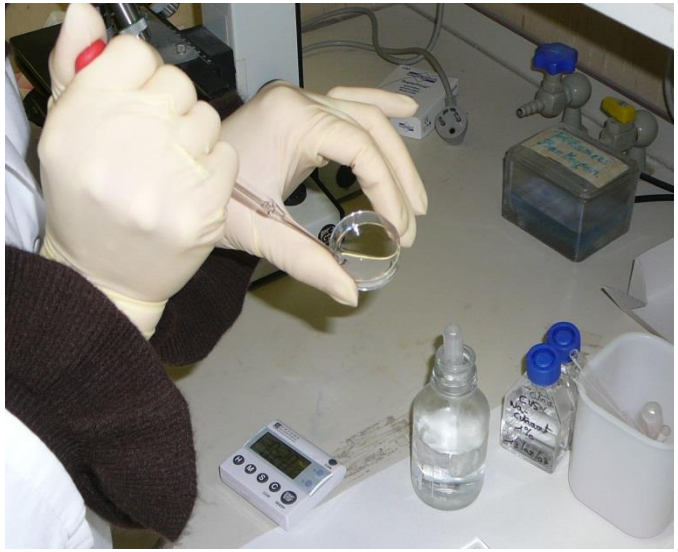


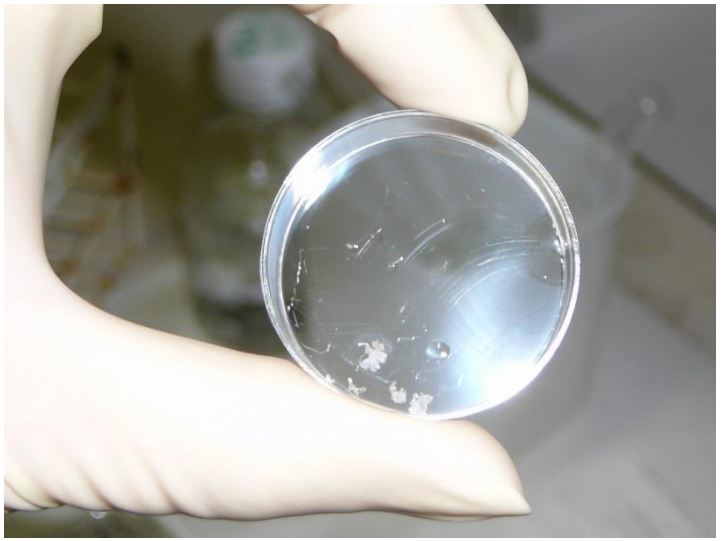
Microscopic dissection chorionic villi

1 villi (uncultured): array CGH + MCC/rapid aneuploidy (QF-PCR)

1 villi: if necessary for DNA/stock

1 villi -> short-term culture (overnight) for FISH + back-up culture (long-term, > 1 week)

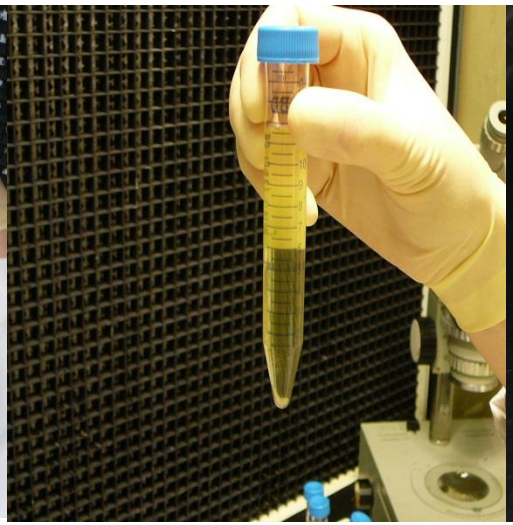




Prenatal culture room-AC

1 tube (10 ml): array CGH + MCC/rapid aneuploidy (QF-PCR)
1 tube: : if necessary for DNA/stock

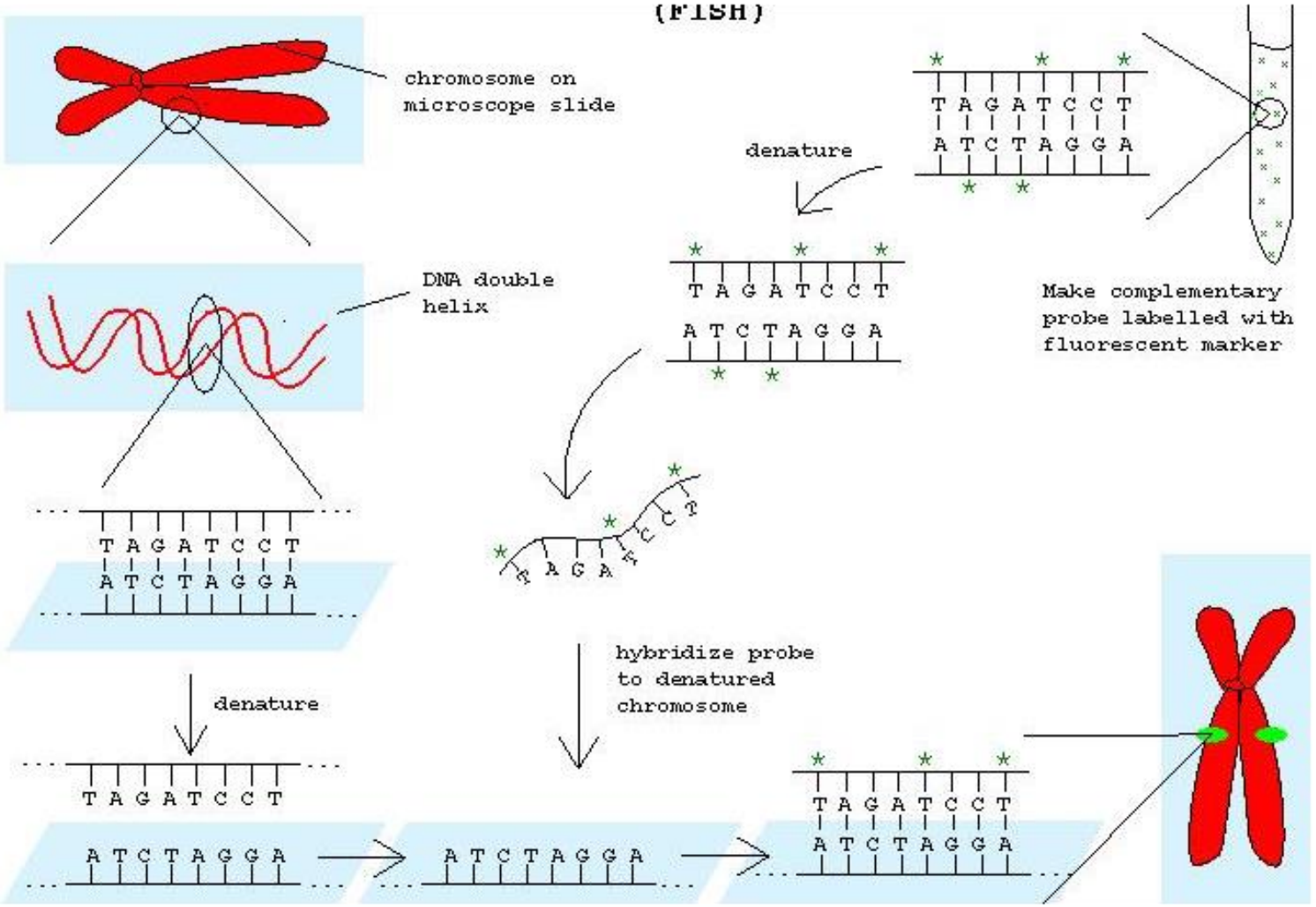
1 tube: FISH (3 ml) +
back-up culture (7 ml)



pellet

Washing

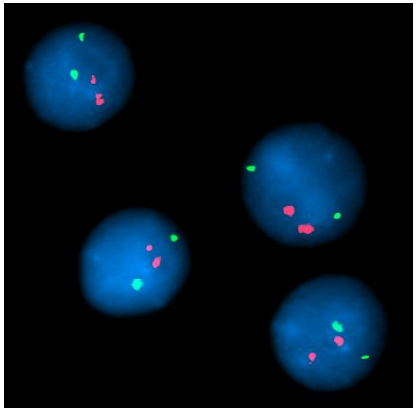
Fluorescence in situ hybridisation



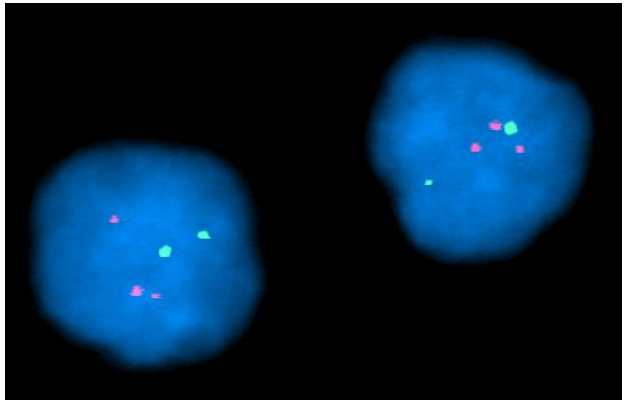
Fluorescent in situ hybridisation

Aneuploidy screening (interphase nuclei: direct test)

- X,Y,13,18,21

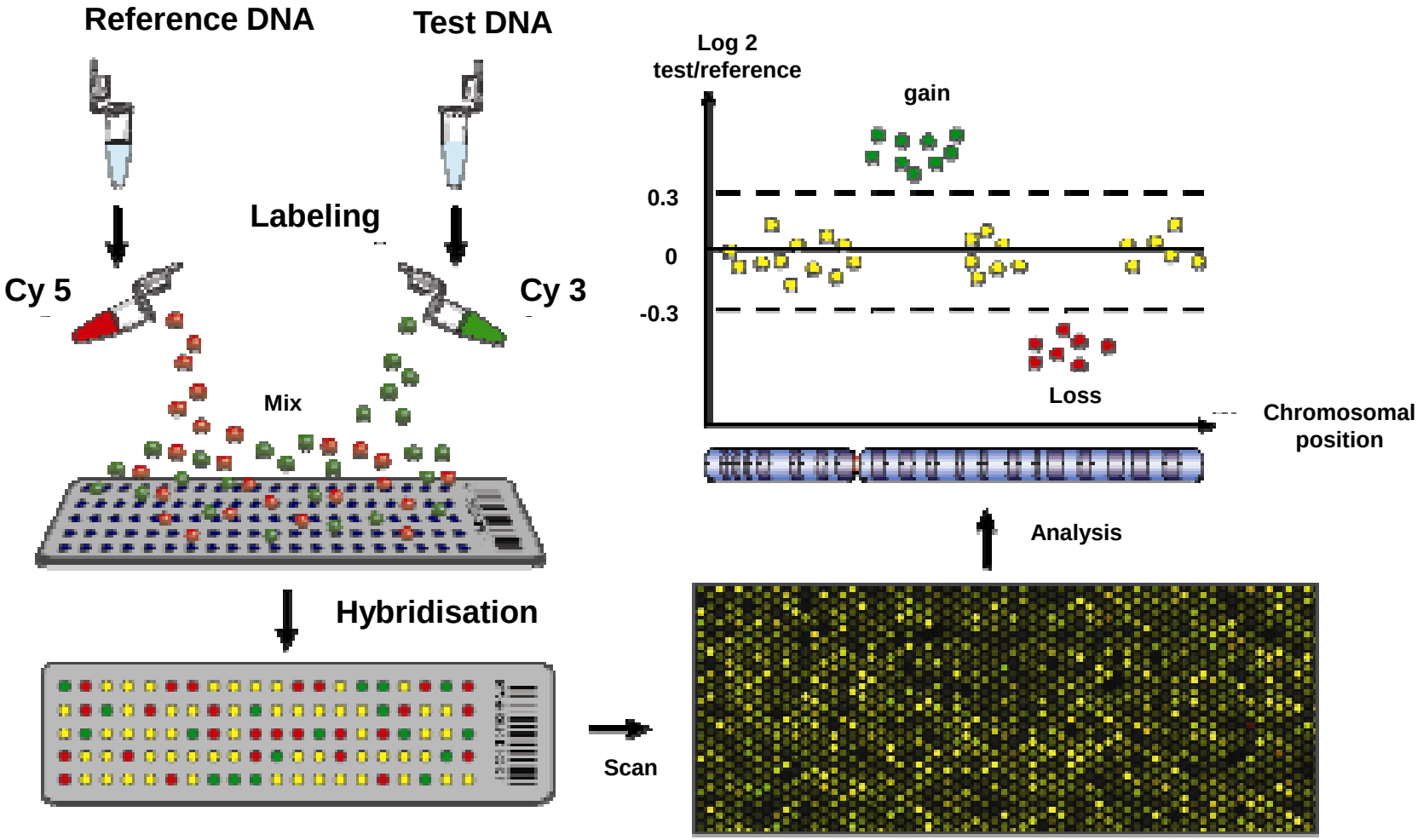


Normal result
(XX, 21)



Trisomy 21 (XX)

Array CGH-Principal



Array CGH prenatal result

- In Belgium 2013: aCGH for all prenatal samples
  - → consensus: to use 60K arrays (60 000 probes) or an equivalent for an average resolution of 400 kb
  - → Additional diagnostic yield (compared to conventional kayotyping; Shaffer et al. 2012; Wapner et al.2012):
    - ±10% in fetuses with multiple ultrasound abnormalities
    - ± 1% in lower risk women, such as those of advanced maternal age
  - → Drawback: introduce CNVs of uncertainty into the diagnostic interpretation

National consensus guideline between the 8 Centres for Medical Genetics in Belgium

- Practical recommendation of pre- and post-counselling
  - → can we expect parents to make ‘on spot’ decisions on what they do and do not want to know?
  - → should we confront parents with questions that are unlikely to be relevant for them?
- How to interpret and report prenatal array results

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Review

Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges

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Prenatal array guidelines

- Classification of variants with regard to pathogenicity:
  - → Pathogenic
  - → Benign variants without functional consequences
  - → Unclassified variants (UV)

https://www.college-genetics.be/assets/recommendations/fr/guidelines/BeSHG%20prenatal%20consortium_guidelines%20prenatal%20array.pdf

Pathogenic CNV

- known to be associated with a phenotype (e.g. del22q11.2)
- resulting in a known effect on gene function and known phenotypic effect

Are communicated

Benign CNV without functional consequences

- **Is repeatedly found in the normal population and not enriched in individuals with abnormal phenotypes**

Are NOT communicated

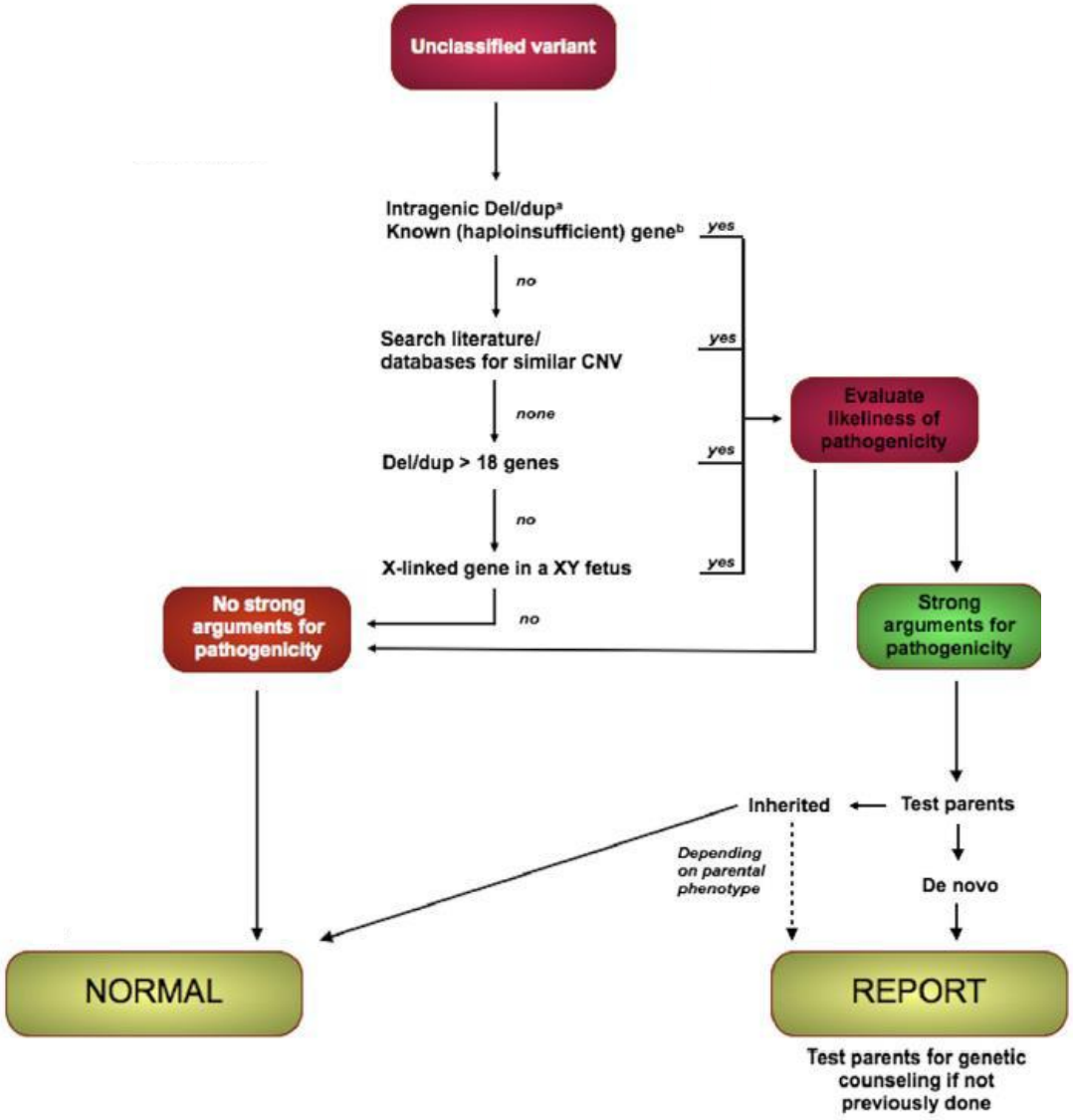
Unclassified variants (UV)

- In principle, UVs are NOT communicated and parental analysis is not performed.
  - unless one expects that this will add to the interpretation of the UV and to the decision to communicate this CNV.

Examples include CNVs with a higher degree of suspicion that they may cause a phenotype, the presence of ultrasound anomalies, family history etc.

In case of uncertainty, the ad hoc committee is consulted for advice. This is done before the final protocol is issued.

Analysis prenatal arrays



SUSCEPTIBILITY CNVs

- CNVs that are risk factors for developmental disorders

NOT communicated

- unless the risk is large enough and/or the CNV is associated with structural malformations for which ultrasound follow-up is indicated

SEE list

available on the website of the College for Genetics: https://www.college-genetics.be/nl/voor-deprofessionele/good-practice-et-richtlijnen-voor-beroepsbeoefenaars/richtlijnen.html.

List of susceptibility loci

chr	start in Mb (hg19)	stop in Mb (hg 19)	size in kb	CNV	gene	phenotype	morph. anomaly	return?	OMIM	update May 2017
1	146,57	147,39	820	distal 1q21.1 dup	*GJA5 (CX40)*	ID, DD, ASD, schizophrenia	macrocephaly, CHD	YES	612475	YES
1	146,57	147,39	820	distal 1q21.1 del	*GJA5 (CX40)*	ID, DD, ASD, SZ, facial dysmorphism	microcephaly, CHD, renal and urinary tract anomalies	YES	612474	YES
1	171,81	172,38(?)	57	1q24.3 del	*DNM3*	ID	IUGR, microcephaly, brachydactyly	YES		
15	31,13	32,48	1350	15q13.3 del	*CHRNA7*	DD, ID, ASD, epilepsy, SZ	microcephaly, CHD	YES	612001	YES
15	99,36	102,52	3160	15q26 del	*IGF1R*	MR	IUGR	YES		YES
16	28,74	28,96	220	16p11.2 distal del	*SH2B1*	obesity, DD, ID, SZ	none	YES	613444	YES
16	29,59	30,19	600	16p11.2 proximal dup	*TBX6*	ASD, ID, DD, SZ, anorexia	microcephaly	~~NO~~ YES	614671	moved to YES since actionable; penetrance del and dup comparable
16	29,59	30,19	600	16p11.2 proximal del	*TBX6*	ID, DD, ASD, obesity, SZ, speech delay	macrocephaly, vertebra	YES	611913	YES
17	34,82	36,21	1390	17q12 deletion syndrome RCAD (renal cysts & diabetes)	*TCF2*	facial dysmorphy, genital abnormalities, ID, DD, ASD, MODY	renal anomalies	YES	614527	YES
22	19,02	20,29	1270	22q11.2 dup	*TBX1*	ASD, ID, DD, dysmorphic features	microcephaly, CHD	YES	608363	YES
1	144,97	146,61	1640	1q21.1 dup	*HFE2*	DD, ASD	CHD	NO		NO
2	50	51,11	1110	2p16.3 del	*NRXN1*	ID, ASD, SZ, DD, dysmorphic features	none	NO	614332	NO
2	110,87	110,98	110	2q13 dup	*NPHP1*	ASD, ID	none	NO		NO
3	197,2	198,84	1600	3q29 dup		MR, DD	none	NO		NO
13	20,81	21,01	1200	13q12 dup	*CRYL1*	?	?	NO		NO
15	22,8	23,09	290	15q11.2 dup	*NIPA1*	DD, motor delay, speech delay, ASD	none	NO		NO (likely benign)
15	22,8	23,09	290	15q11.2 del	*NIPA1*	ID, DD, epilepsy	CHD	NO	615656	NO (likely benign)
15	31,13	32,48	1350	15q13.3 dup	*CHRNA7*	ADHD, ID, DD, ASD	none	NO		NO (likely benign)
16	14,98	16,48	1500	16p13.11 dup	*MYH11*	ID, ASD, SZ, ADHD	aorta dilatation	NO		NO
16	14,98	16,48	1500	16p13.11 del	*MYH11*	ID, DD, ASD, epilepsy	microcephaly	NO		NO
16	21,94	22,46	520	16p12.2 dup	*EEF2K, CDR2*	?	?	NO		NO (likely benign)
16	21,94	22,46	520	16p12.2 del	*EEF2K, CDR2*	DD, speech dealy	cranofacial and skeletal abnormalities, CHD	NO	136570	NO

Incidental findings

- Only highly penetrant monogenic disorders are considered, with validated evidence on the phenotype associated with the deletion or duplication

Incidental findings

Four categories are distinguished:

- **Late-onset genetic disorders with clinical utility**

➢ will be communicated (typically cancer caused by the deletion of a tumor suppressor gene)

- **Late onset disease without therapeutic possibilities**

➢ the decision after consulting the ad hoc committee

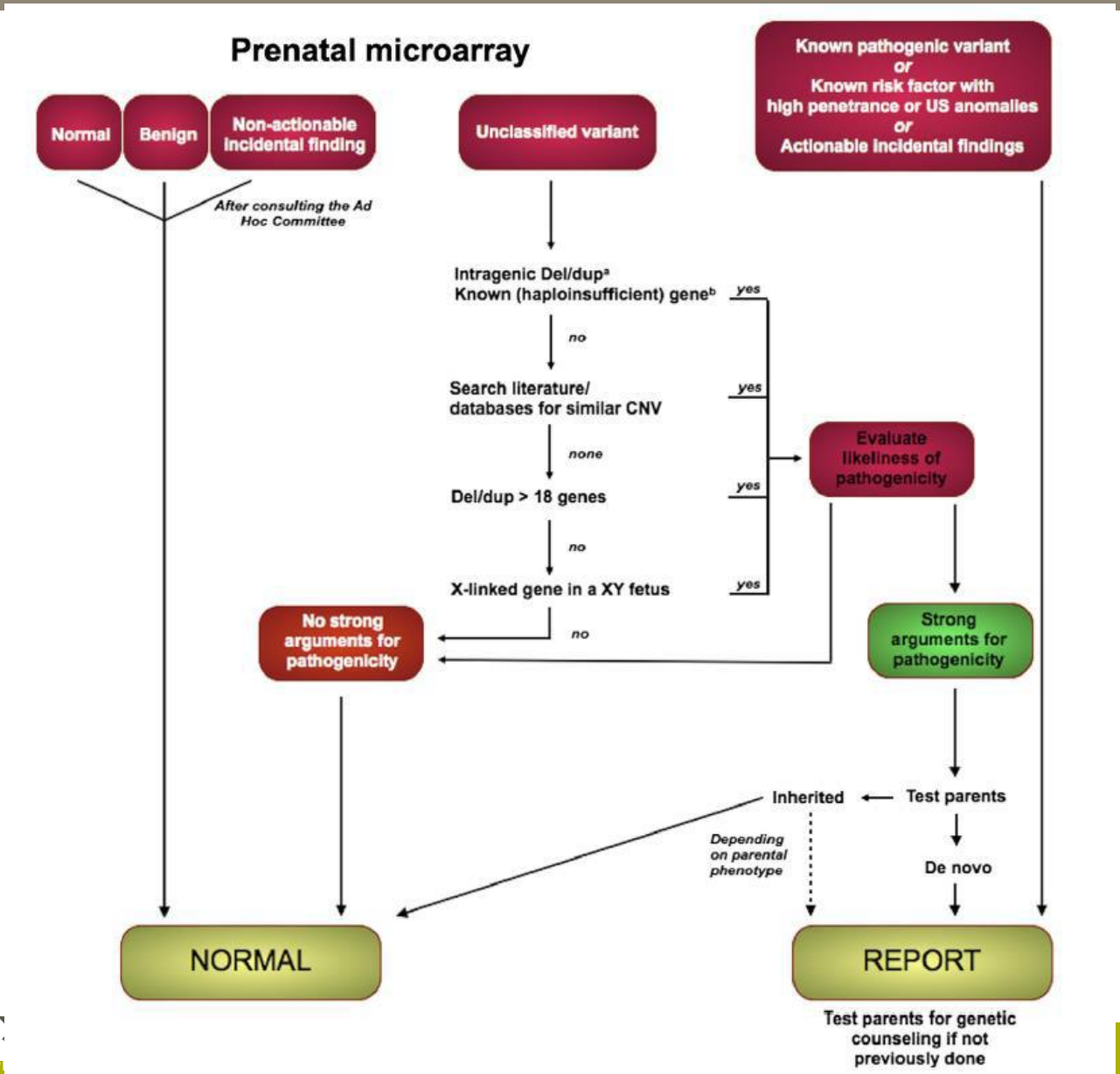
- **Carrier for X-linked recessive disorders**

➢ will be communicated

- **Carrier for autosomal recessive disorders**

➢ will not be communicated

Analysis prenatal arrays



Implementation of an *Ad Hoc* committee

- 2 clinical geneticists and 2 cytogeneticist from each center = 32 individuals
- cases are presented to the committee through e-mail
- AIM: to reach a consensus decision within 24-48h

- less subjective
- more consistent counselling in case of second opinion in another centre
- rapid learning curve on evaluation of 'difficult' CNVs

Advisory role

Clinician holds responsibility on final decision

To Do / Ongoing national guidelines

- **Regular re-evaluation to further optimize the consensus approach**

- **Address several outstanding questions**
  - **proportion of cases with unclassified variant?**
  - **% detection of causal CNVs in different indications?**
  - **% of incidental findings?**
  - **how often is parental analysis indicated?**
  - **incidence of susceptibility loci?**
  - **detection of causal CNVs postnatally?**
  - **postnatal follow-up**

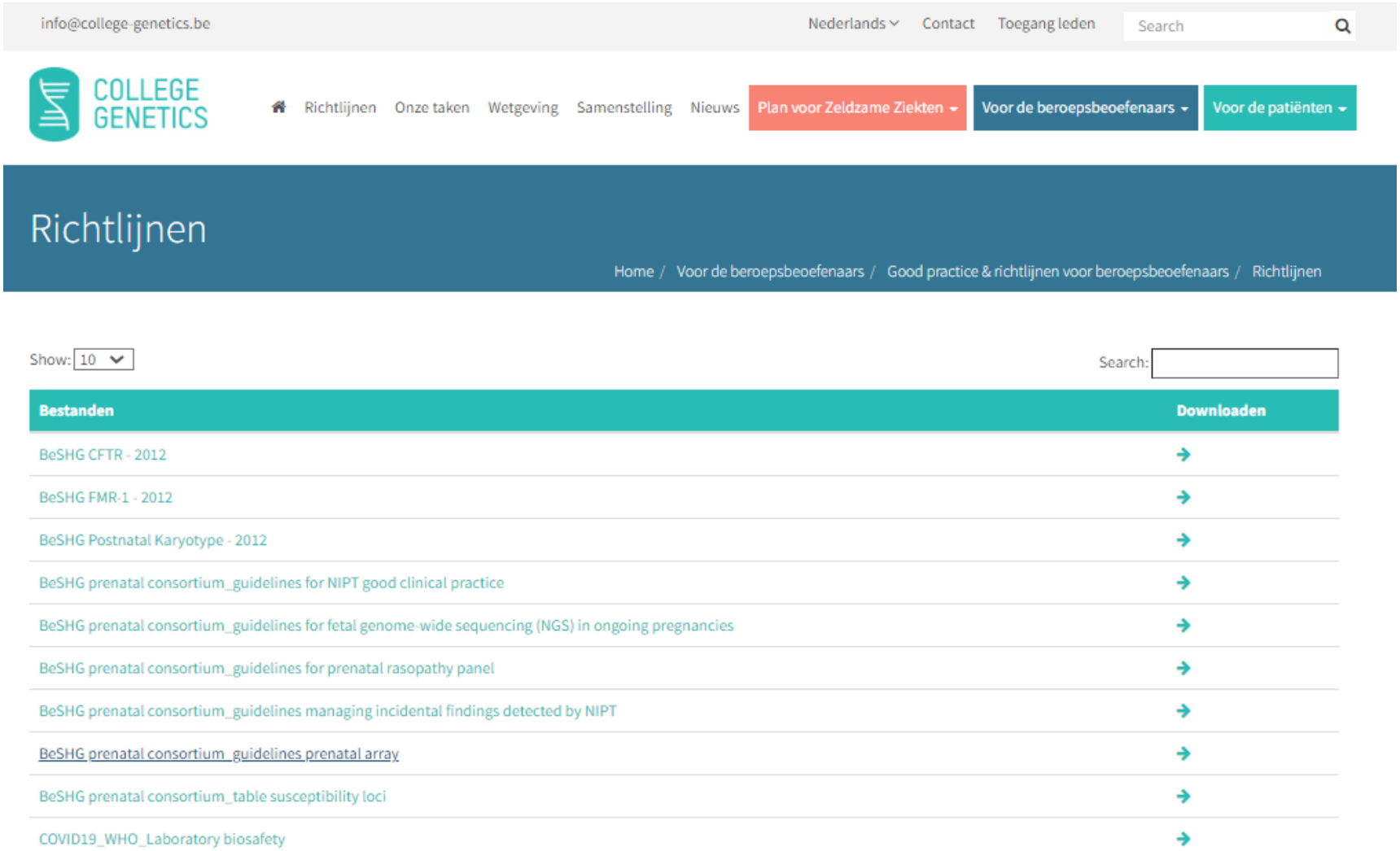
Conclusion national guidelines

- **The National consensus approach solves:**

- **technical issues (resolution, what to test for, etc..)**
- **variation in interpretation amongst laboratories**
- **variation of reporting**
- **issues related to liability**

Practical aid for those routinely using prenatal arrays

Conclusion national guidelines



Mosaicism in prenatal diagnosis

- **Mosaicism**
  - → **Is difficult for making a conclusion**
  - → **The presence of two or more cell lines in a tissue sample**
  - → **Three categories**
    - **Confined placental mosaicism**
    - **True Constitutional fetal mosaicism**
    - **Pseudomosaicism refers to an abnormality that arose during tissue culture in vitro (cultural artifact)**

Confined placental Mosaicism

- **Confined placental mosaicism**
  - → **An abnormal cell line may only exist in the extra-embryonic tissues of the placenta**
  - → **Is encountered at CVS rather than AC**
  - → **It is uncommon that mosaicism at CVS reflects a true constitutional mosaicism of the fetus**
    - **More than 50000 procedures (grati et al. 2014)**
      - **In 2,2% of CVS mosaicism was seen -> 0,3% proved to have true fetal mosaicism**

True fetal Mosaicism?

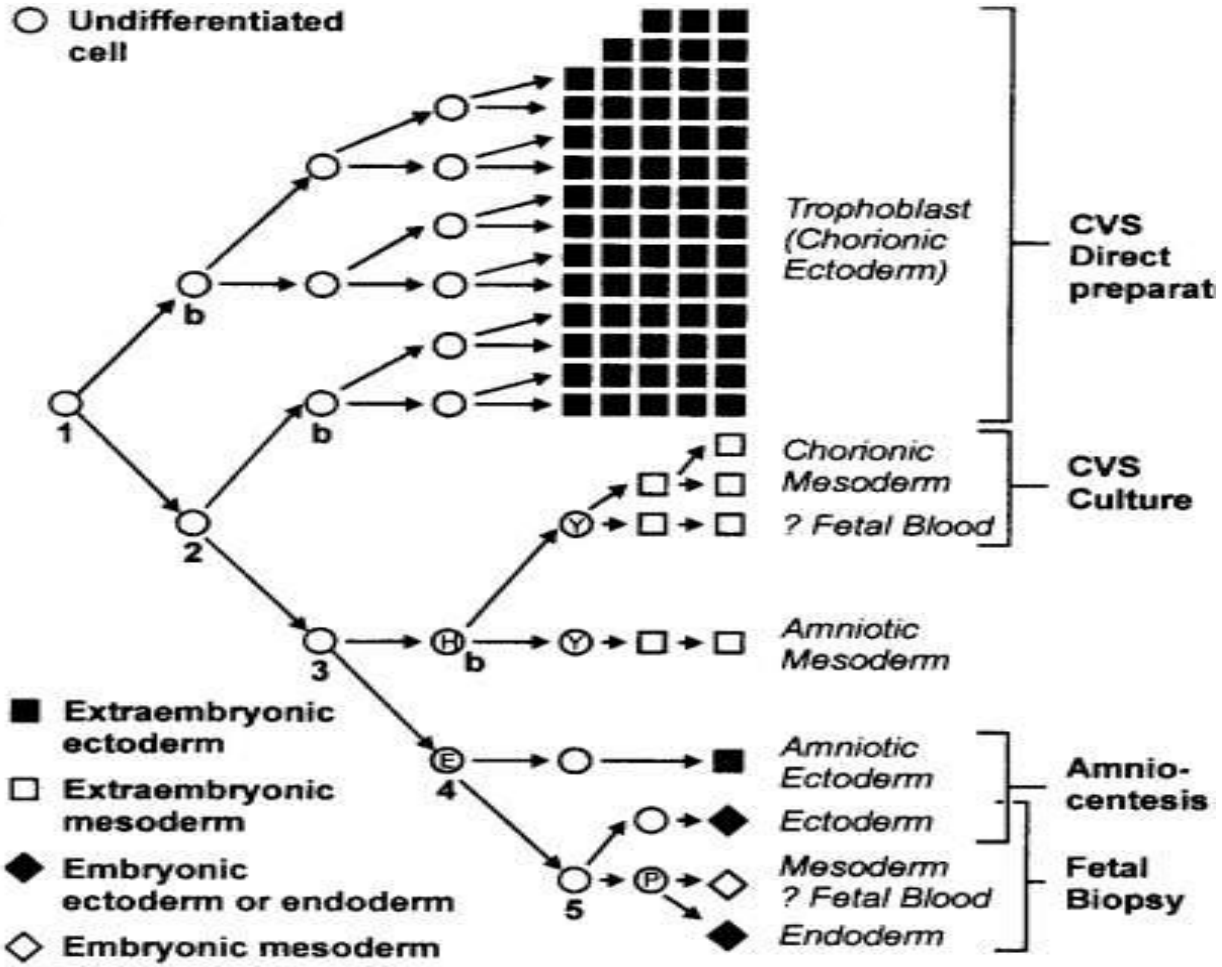
- **Chorion Villi Sampling**

  → **Samples more distantly related from the fetus**

- **Amniocentesis**

  → **Cells closely reflect the true constitution of the fetus**

Embryological Origins



Gardner & Sutherland 3rd Edition 2004