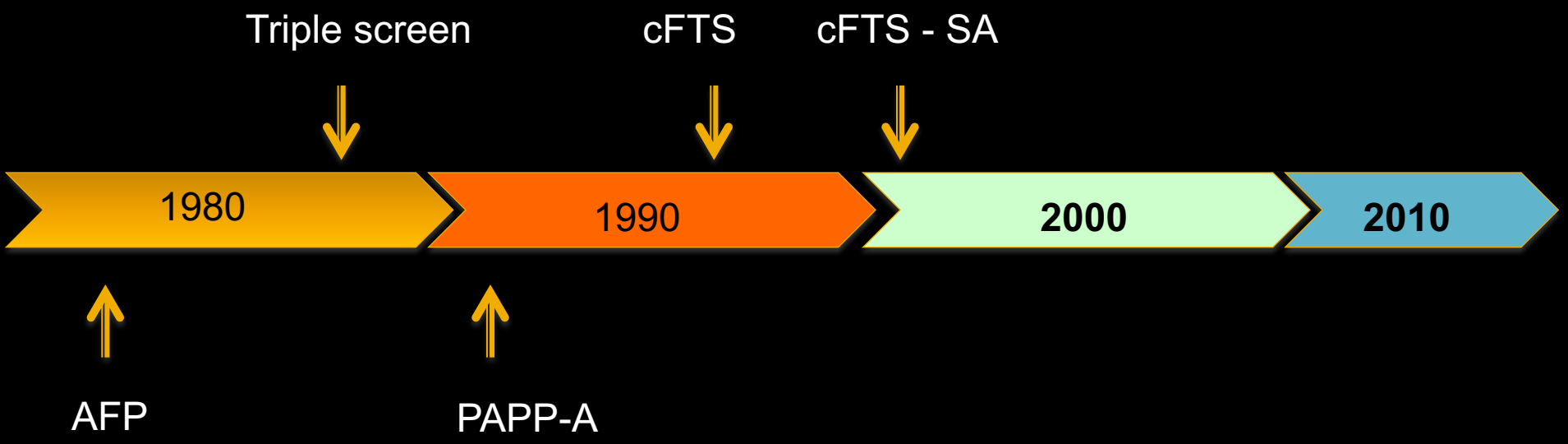
Dr Peter Muller
Maternal-Fetal Medicine
Women's and Children's Hospital

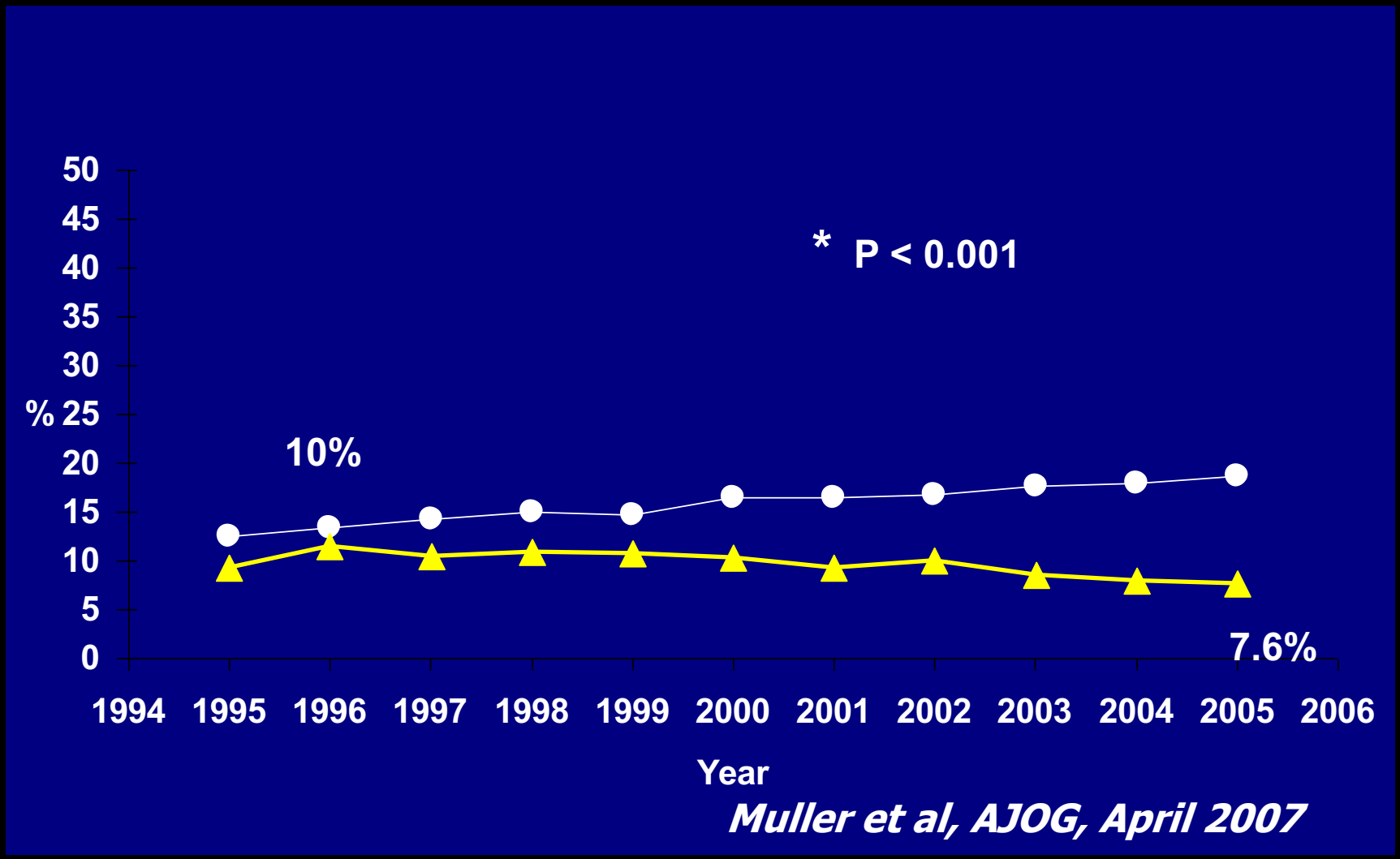
Avoid the Quick Sand – Aneuploidy Screening Common Questions

Never know what you are going to find!

History



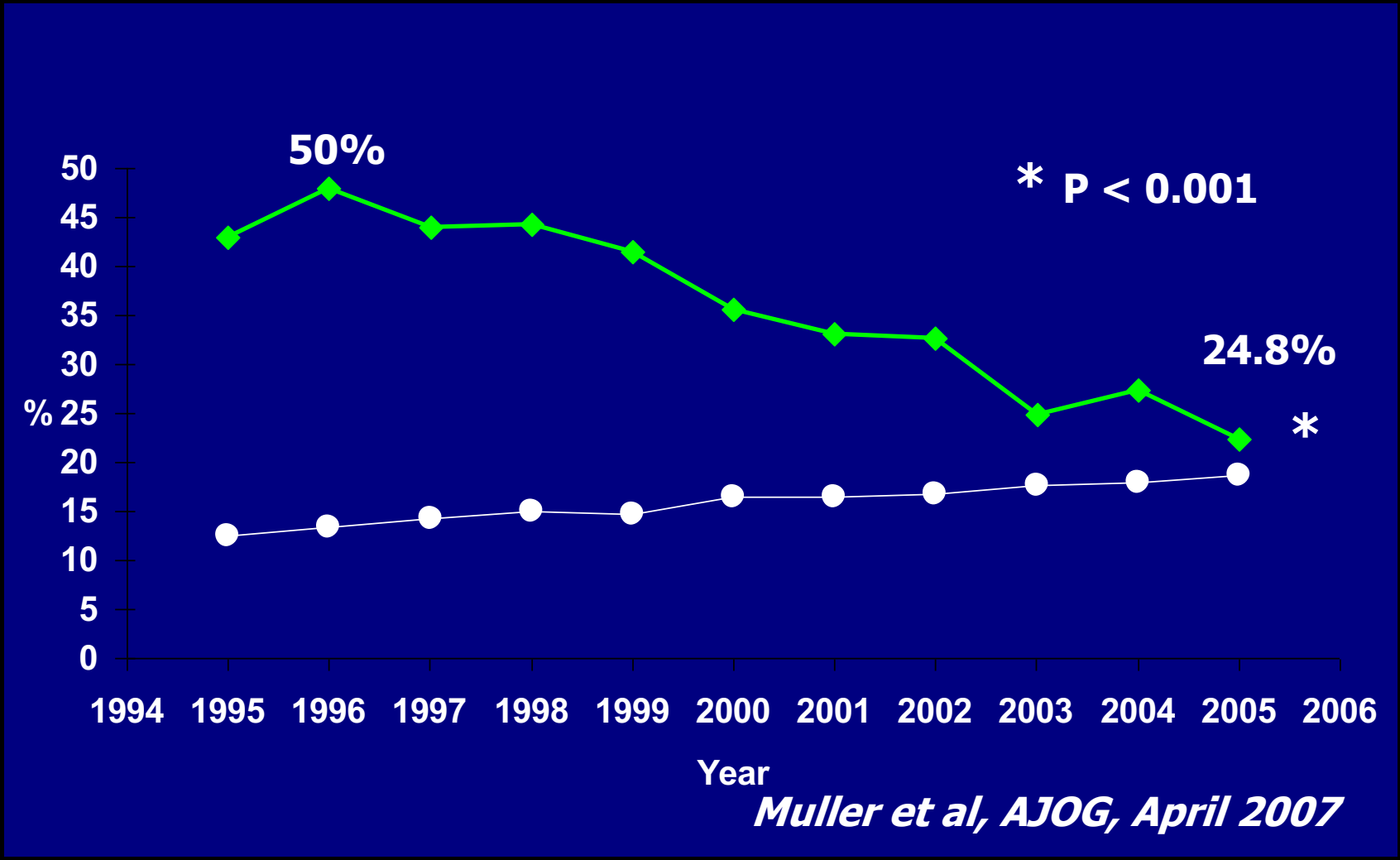
% of confinements undergoing invasive prenatal tests (Δ)



Muller et al, AJOG, April 2007

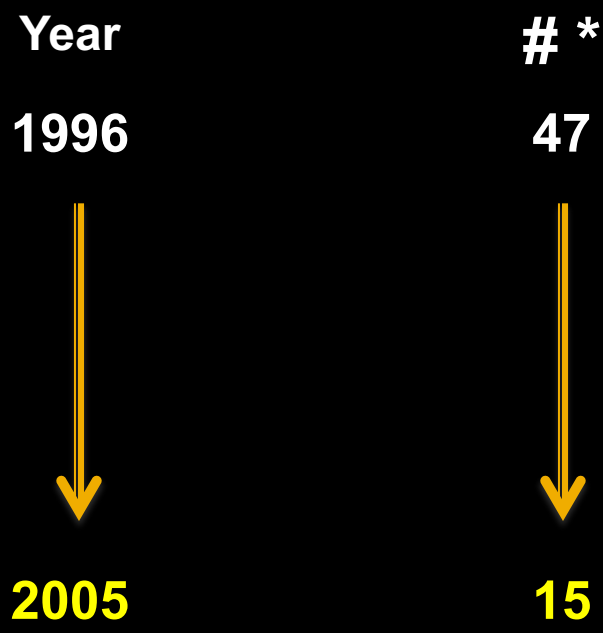
AMA

undergoing invasive prenatal tests (◊)



Muller et al, AJOG, April 2007

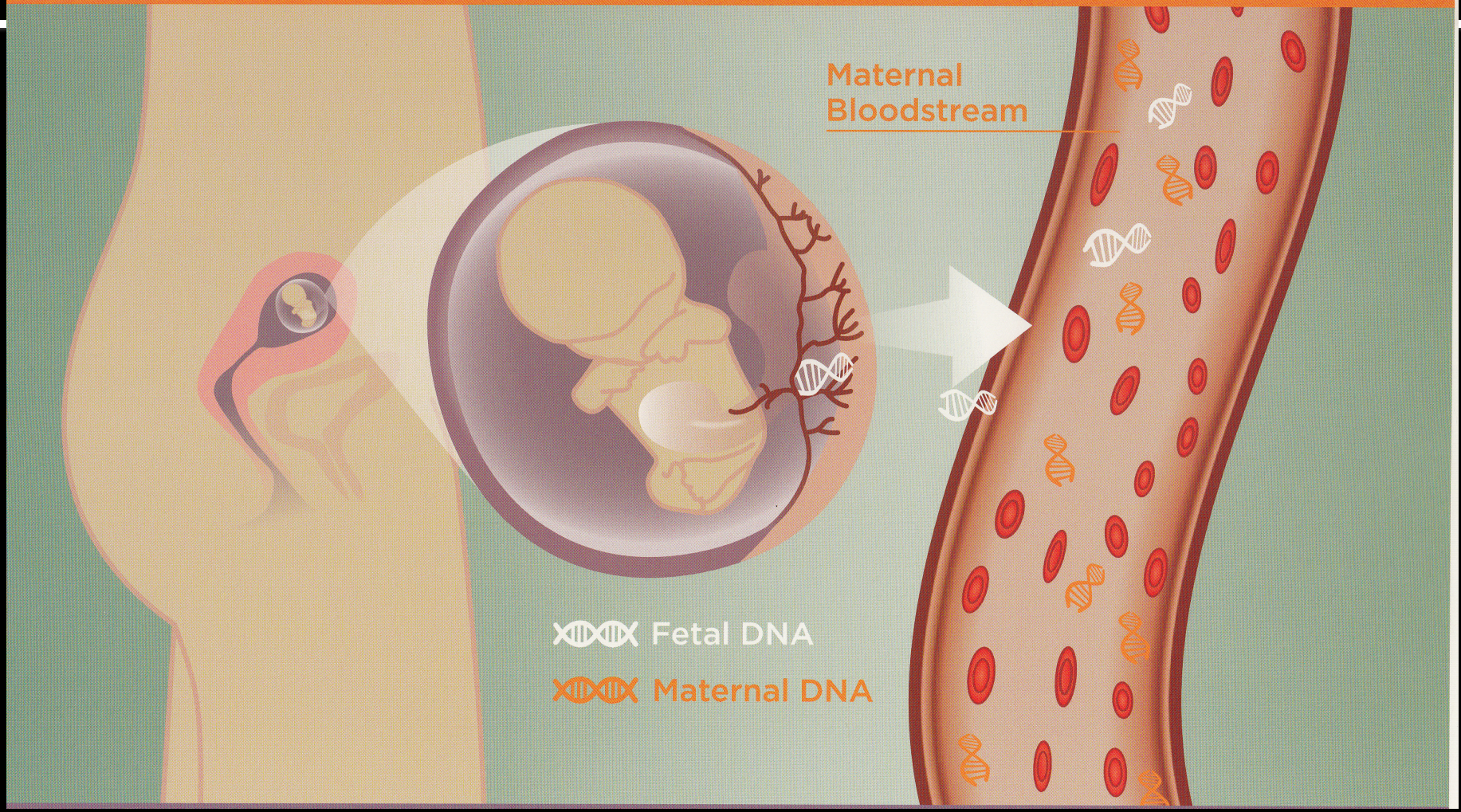
Number of invasive prenatal tests to diagnose one aneuploid fetus



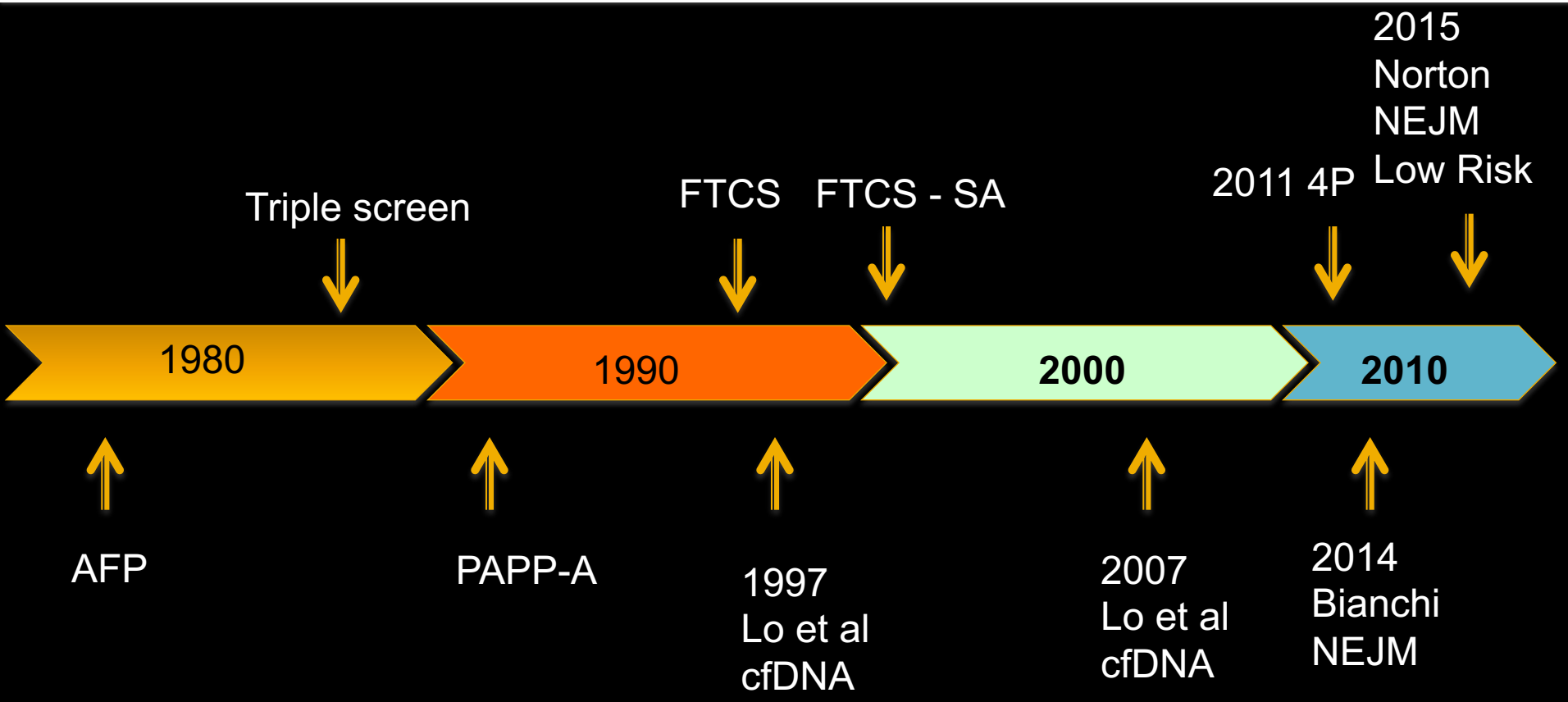
* p < 0.001

Muller et al, AJOG, April 2007

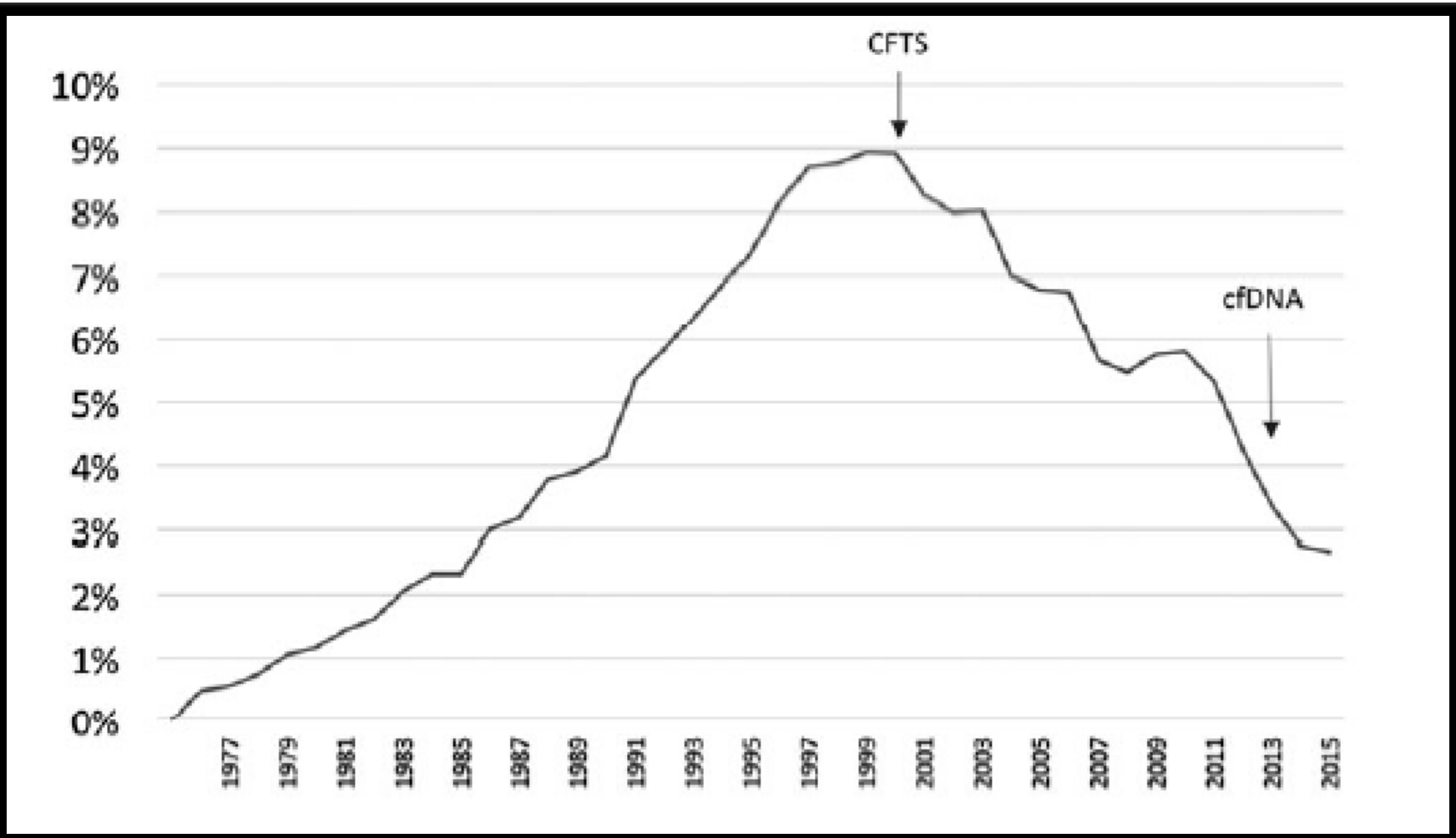
What is Cell-Free DNA?



History - cfDNA

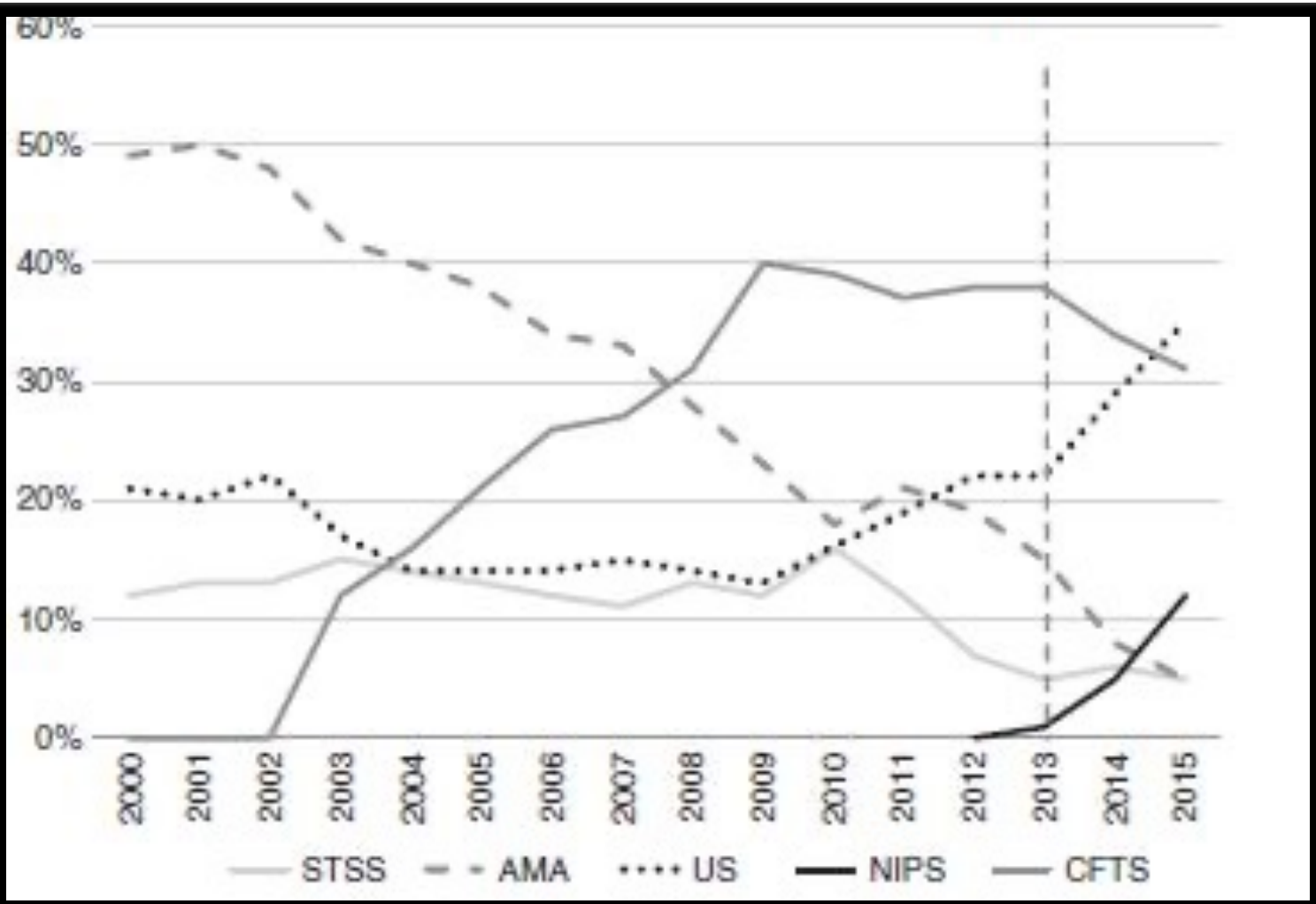


% of births with diagnostic testing Victoria



Hui, L and Norton M, Pren Diag; 2018; 38:246-49

Indication for prenatal test Victoria



Hui L et al Genet Med 2017;19:1138

Victorian Population

- Proportion of births have invasive testing
  - 2.7%
- Yield of invasive tests
  - 20%
  - 1/5 positive invasive test

Hui L et al Genet Med 2017;19:1138

Question 1

- Time to offer NIPT to all women, low or high risk?

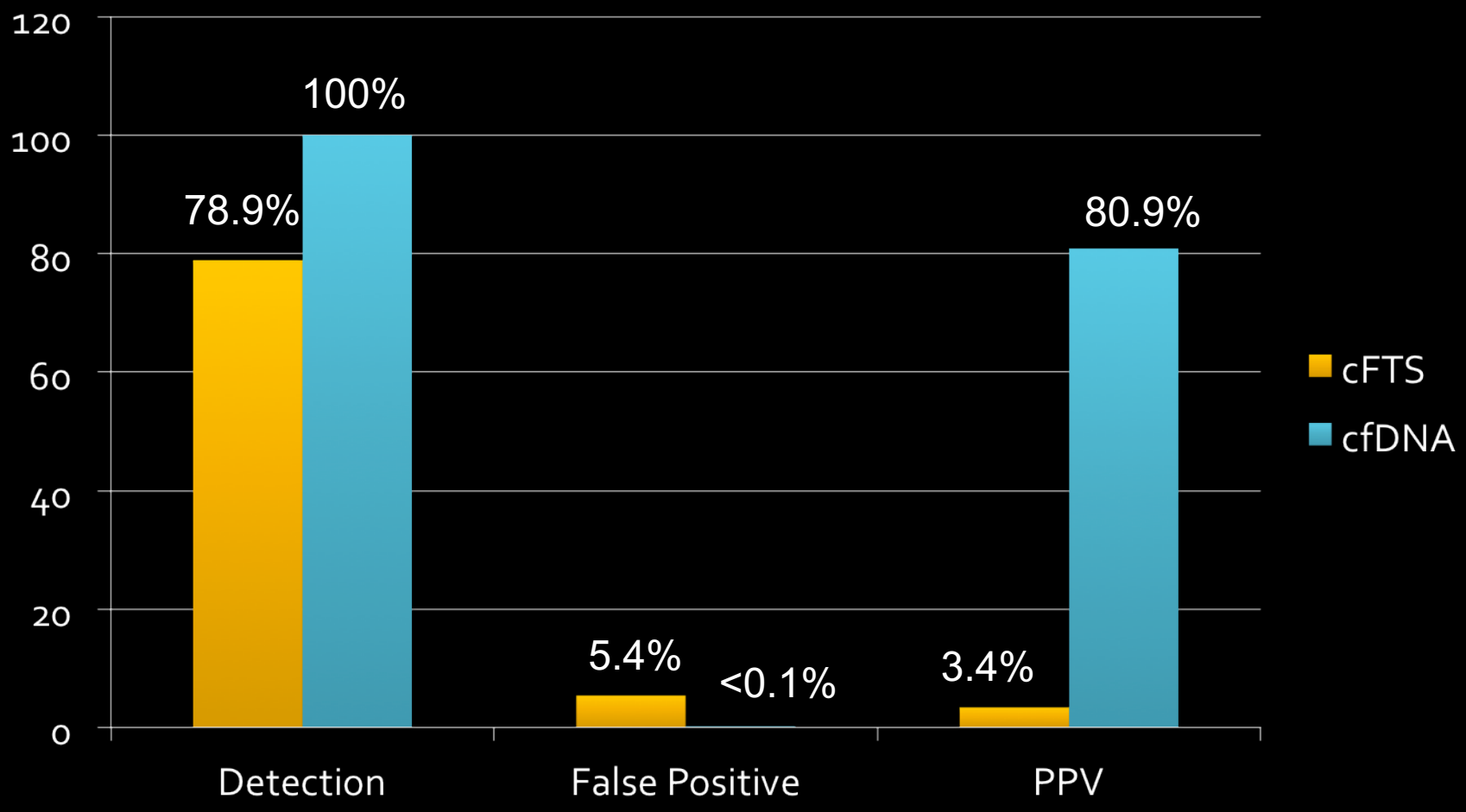
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 23, 2015 VOL. 372 NO. 17

Cell-free DNA Analysis for Noninvasive Examination of Trisomy

Mary E. Norton, M.D., Bo Jacobsson, M.D., Ph.D., Geeta K. Swamy, M.D., Louise C. Laurent, M.D., Ph.D., Angela C. Ranzini, M.D., Herb Brar, M.D., Mark W. Tomlinson, M.D., Leonardo Pereira, M.D., M.C.R., Jean L. Spitz, M.P.H., Desiree Hollemon, M.S.N., M.P.H., Howard Cuckle, D.Phil., M.B.A., Thomas J. Musci, M.D., and Ronald J. Wapner, M.D.

cfDNA vs cFTS



Norton M et al, NEJM;2015

cfDNA vs cFTS

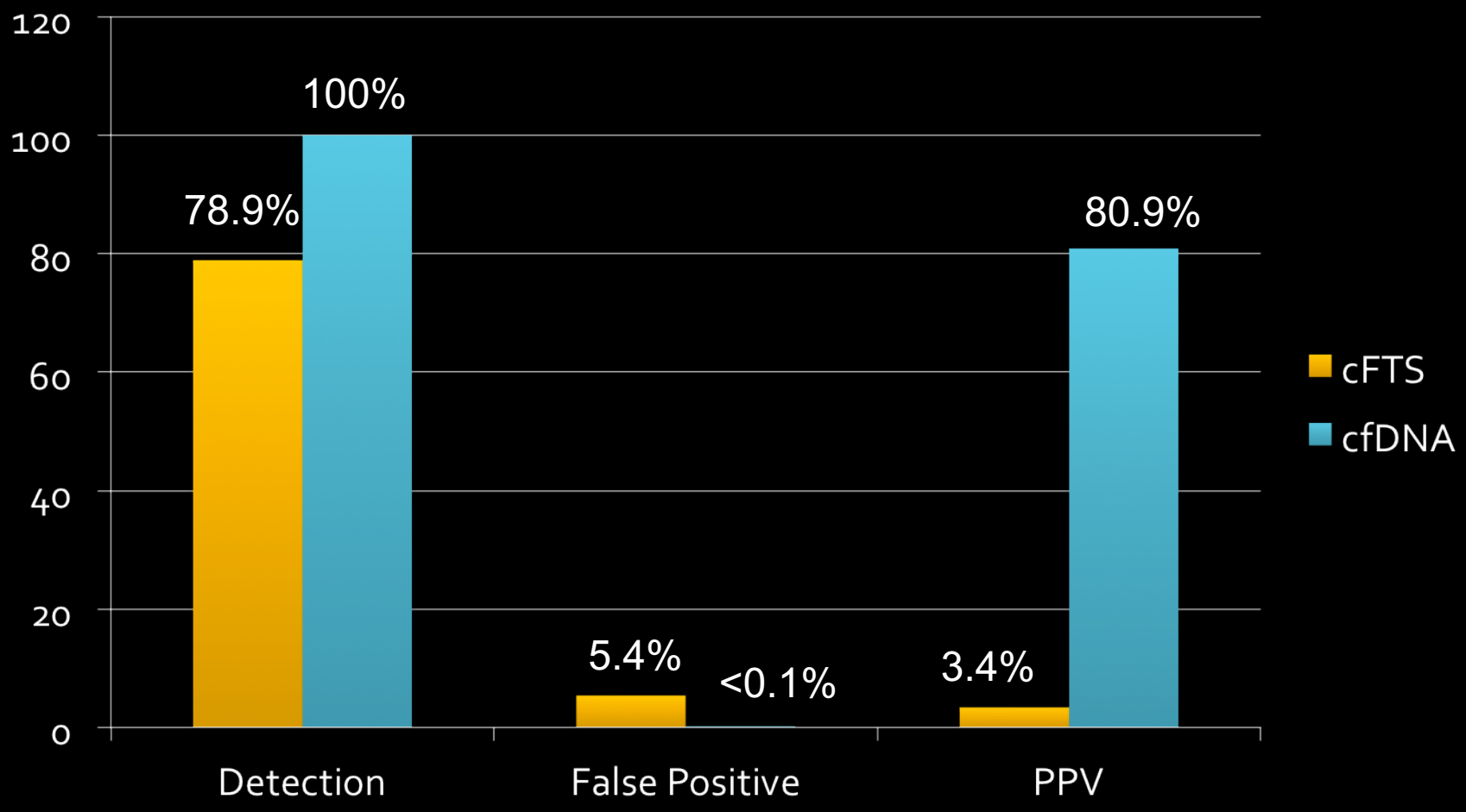
- NIPT false positive 1/10th of multiple marker screening
- PPV with NIPT higher than PPV of FTCS among high risk women

Bianchi DW et al NEJM August 2018

Question 2

- Should both FTCS and NIPT be offered?
- Or Just one?

cfDNA vs cFTS



Norton M et al, NEJM;2015

Both!!

So I will not miss an atypical chromosomal abnormality.

What happens in the "Real world" Victoria

1. Performance of different prenatal screening stratigies
2. Residual risk of major chromosomal abnormality following low risk result.

    1. In other words, what is missed?

Lindquist A et al UOG. 2020:56:215.

Victoria 2015

Screening pathway	Sens T21	Sens T21,T13,T18	Spec T21, T13, T18	Screen Positive
cFTS alone	87.95%	89.57%	97.25	2.94
cfDNA alone	100%	100%	99.93	1.21% 2.42%
STSS	50%	60%	93.17	6.92%

Lindquist A et al UOG. 2020:56:215.

Residual Risk major chromosomal abnormality – low risk screening

Prenatal Screening Pathway	% Risk
Low risk cFTS	0.084% (1:1188) *
Low risk cfDNA	0.13% (1:762) *

* = NS

Lindquist A et al UOG. 2020:56:215.

Conclusion

- Although non-significant difference in residual risk of any major abnormality between cFTS (1:1188) and cfDNA (1:762)
- cfDNA with fewer live born infants with major chromosomal abnormality
- Conclusion:
  - **Do not do both** as a type of screening.

Lindquist A et al UOG. 2020:56:215.

But we need the PAPP-A?????

PAPP-A Level	FGR (BW < 10th%)	PTB < 34 weeks
<5th% (0.4 MoM)	14%	2.3%
< 1st% (0.2 MoM	24%	2.5%

PAPP-A

Biochemistry	Risk of microarray abnormality not detected by NIPT
PAPP-A < 0.2 MoM (<1st%)	4%
BHCG < 0.2 MoM (<1st%)	7%
BHCG > 5.0 MoM (>99th%)	0.5%

Why would you? PAPP-A

- cFTS call back rate
  - 5% overall
  - Close to 20% over 35
  - Close to 25% >= 40
- PAPP-A < 1st% is all we care about
  - 5% call back rate for high Trisomy 21
  - to get a 1% modest risk of SGA < 10th%

College and Society Statements

- RANZCOG – July 2018 - Acceptable first-line screening tests:
- First Trimester Combined Screening
- **OR**
- cell-free DNA (cfDNA)-based screening. patient demographics, and individual patient characteristics.

RANZCOG College Statements & Guidelines July 2018

Question 2?

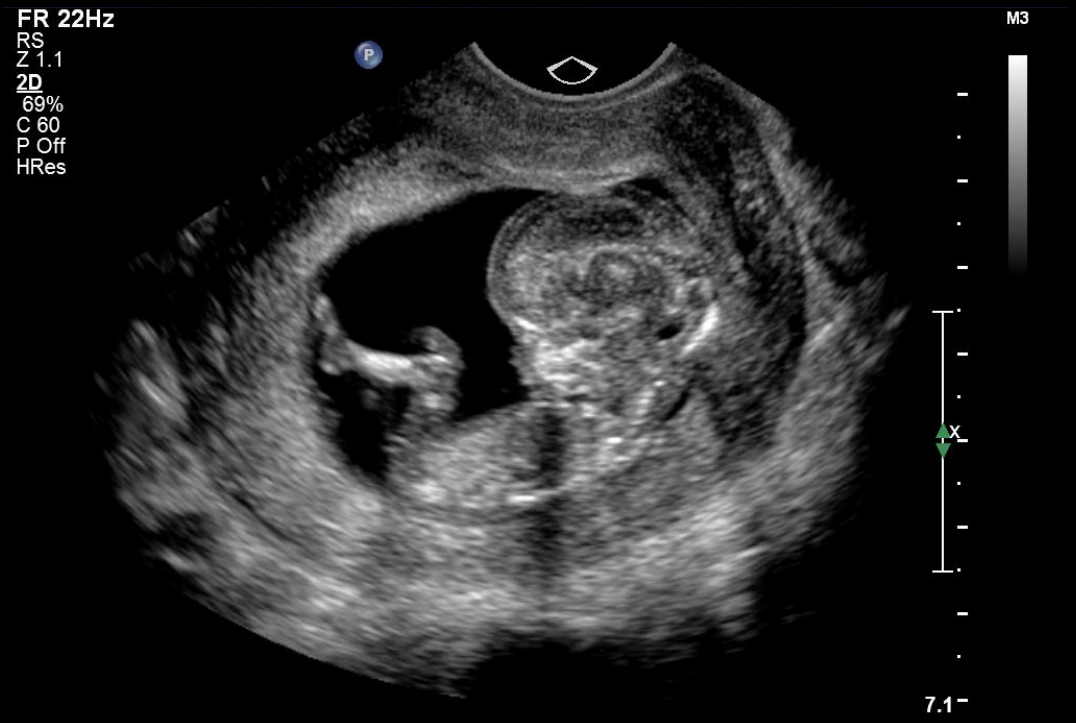
Both as first line?

NO!

Lets Stop it!!

Question 3

- After NIPT, is there still need for First Trimester anatomy ultrasound?



First Trimester Ultrasound Benefits

- Early detection of multiple pregnancy
  - (and probably chorionicity)
- Improved gestational dating
  - Fewer inductions for post dates.

Whitworth M et al, Cochrane Database 2015

- Major fetal abnormalities
  - Early genetic termination of pregnancy

First Trimester Ultrasound Benefits

- Systematic review
  - **51% detection**

Rossi AC et al O&G Vol 122, No. 6, Dec 2013

Low risk vs high risk

- Systematic review
- 32% detection in low risk
- 60% detection in high risk

Karim JN et al, UOG 2016.

Role of 11-14 week scan with negative cfDNA

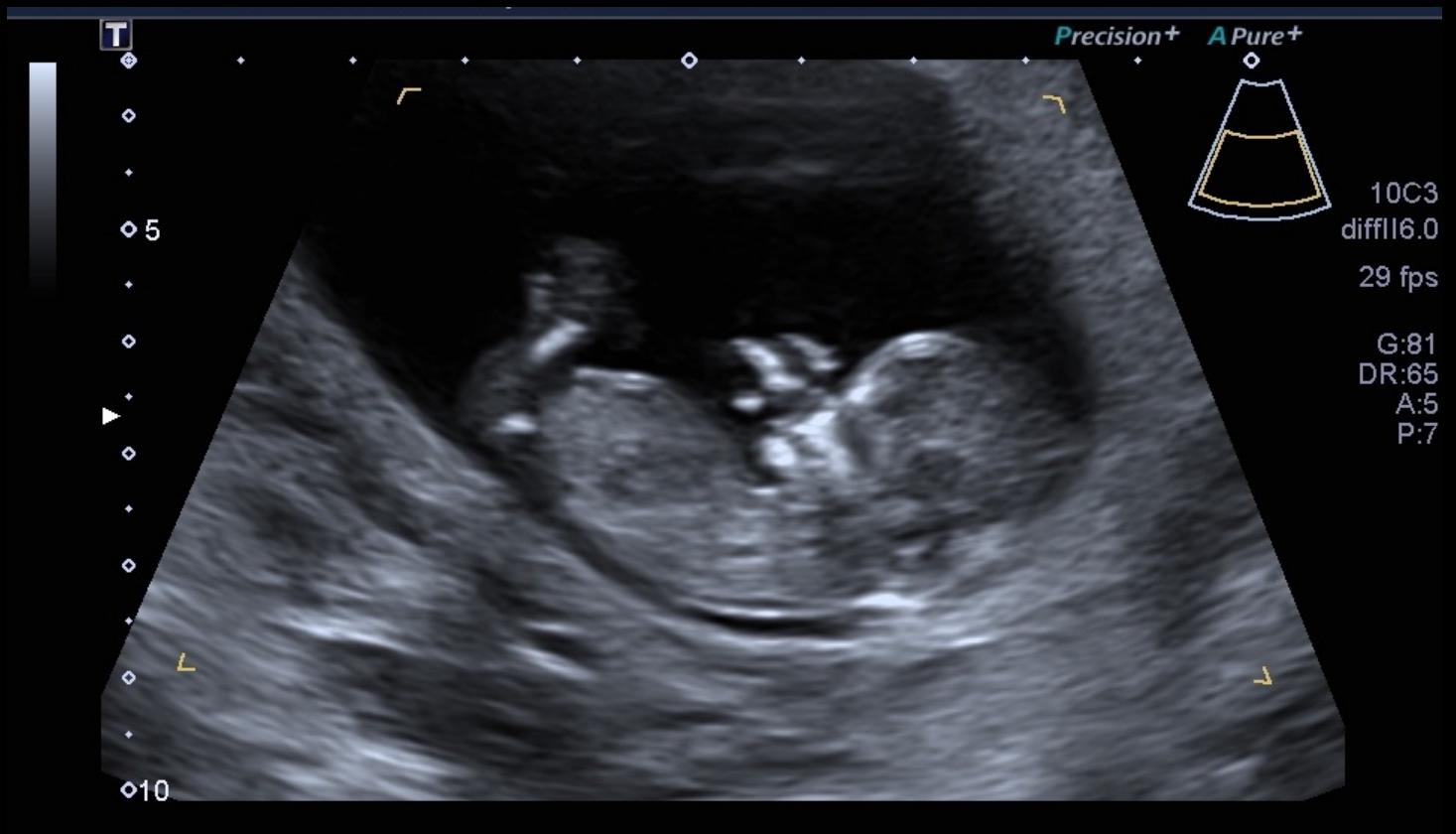
- Negative cfDNA
  - 3.5% had unexpected finding
  - 2.1 with fetal abnormality

Reiff ES et al, Prenat Dx 2016,36,260-5

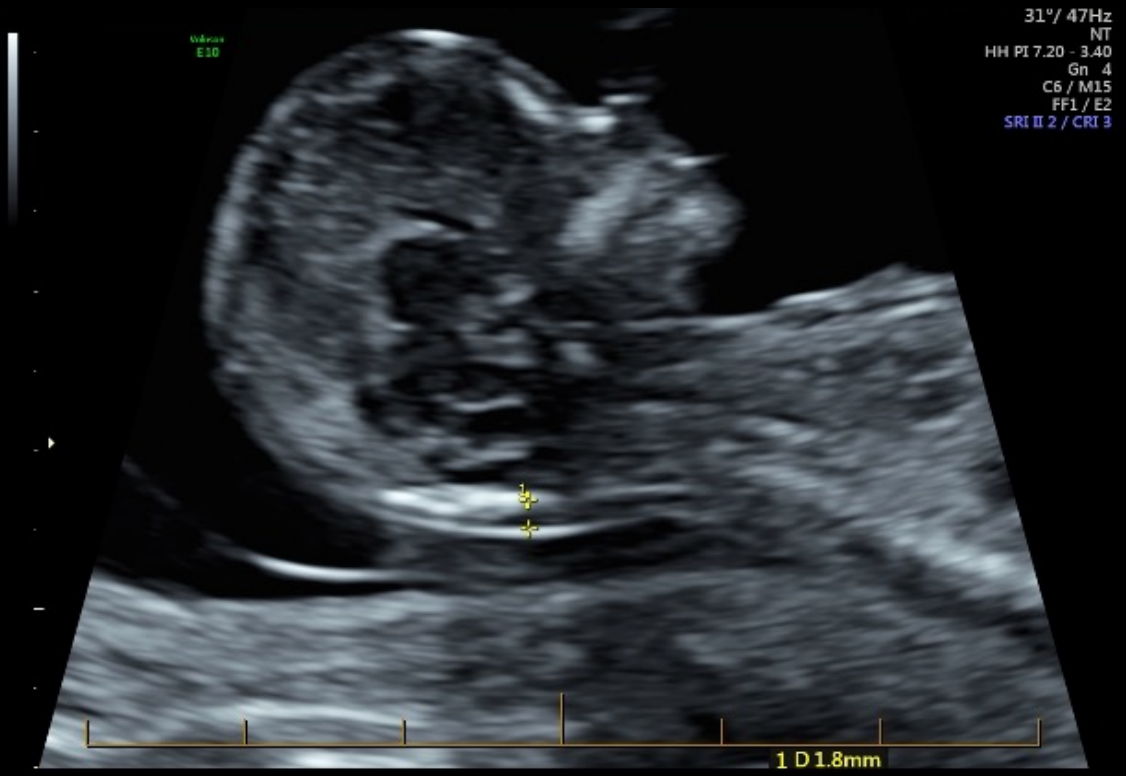
If perform cfDNA, when should we do the ultrasound.

"Best Bang for your buck"

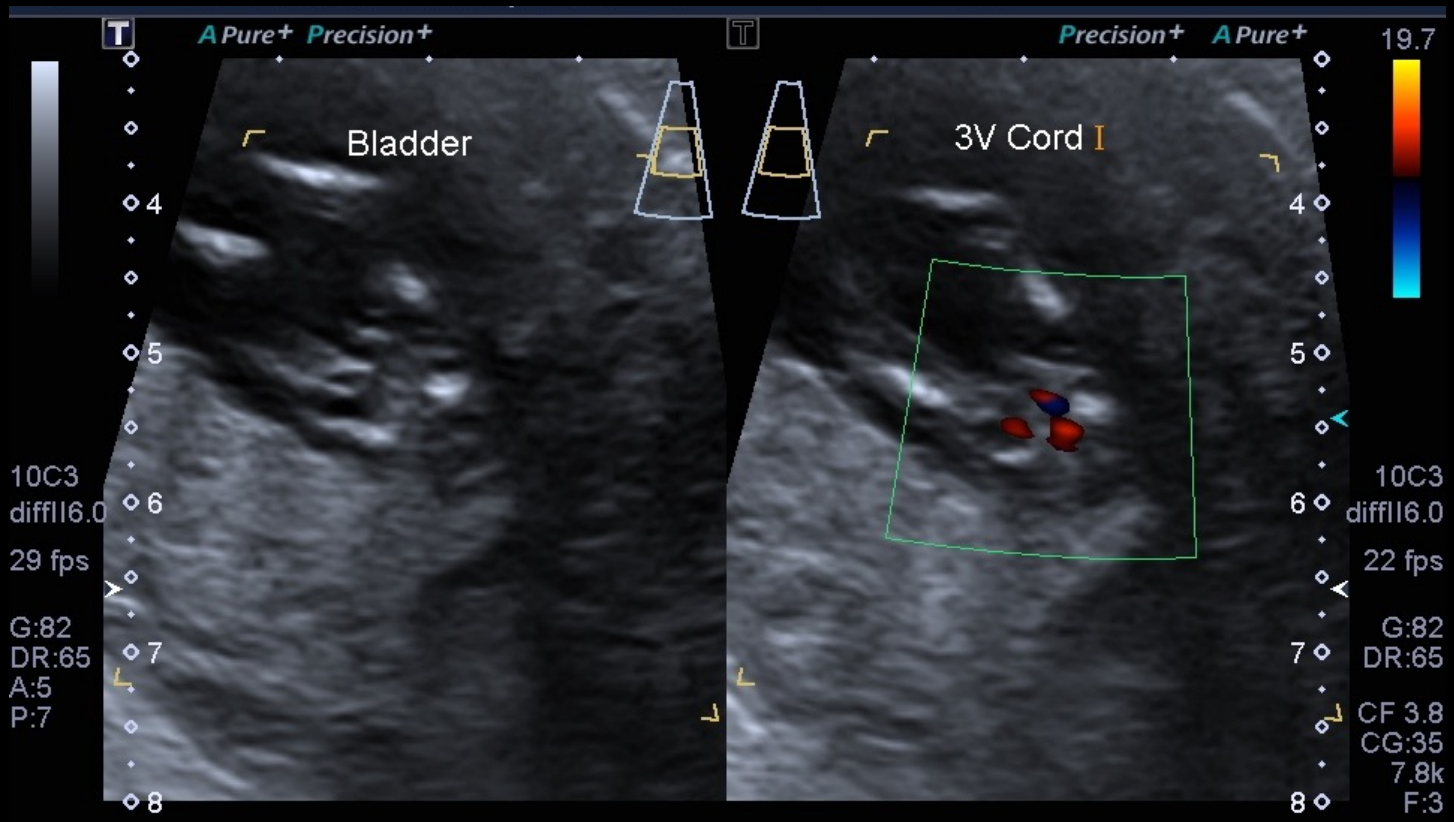
11 + 6 weeks



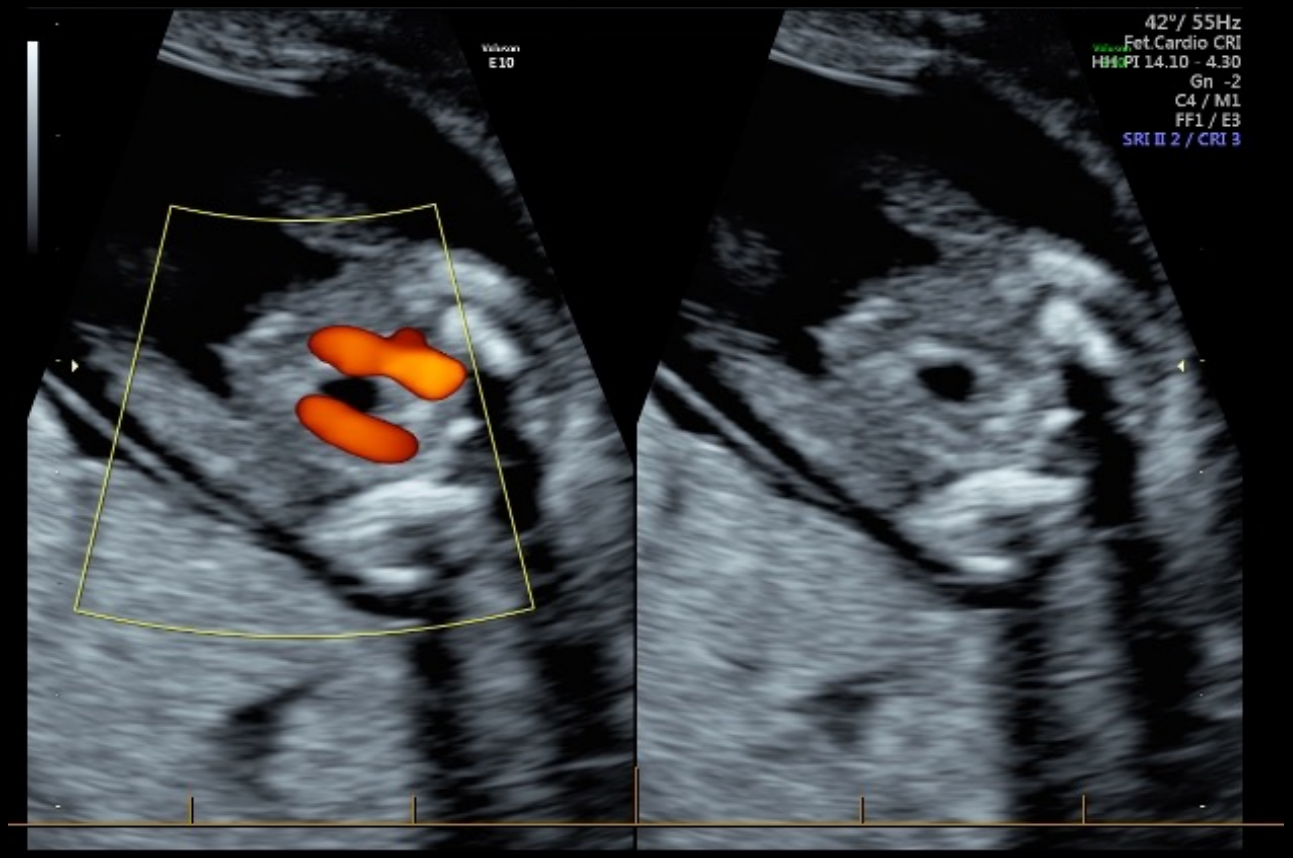
13 + 2 weeks



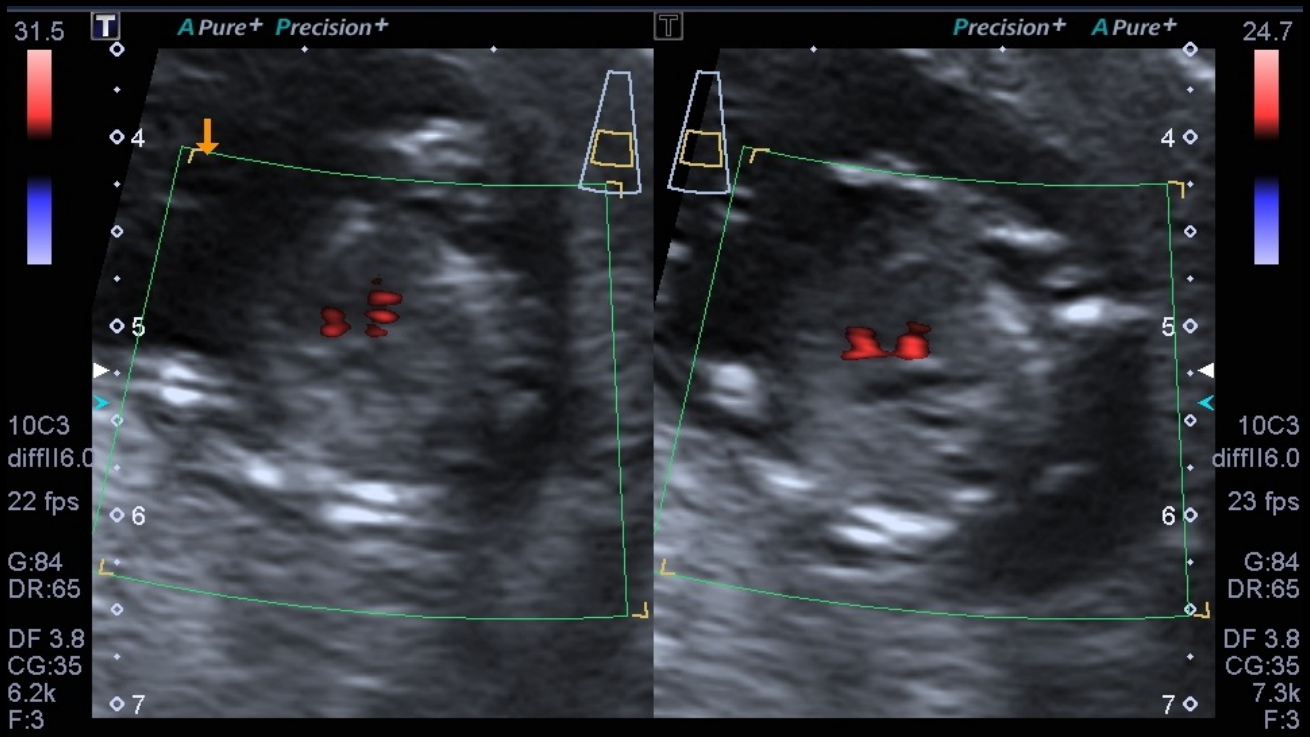
11 + 6 weeks


A Pure+ Precision+
Bladder
10C3
diffII6.0
29 fps
G:82
DR:65
A:5
P:7
Precision+ A Pure+
19.7
3V Cord I
10C3
diffII6.0
22 fps
G:82
DR:65
CF 3.8
CG:35
7.8k
F:3

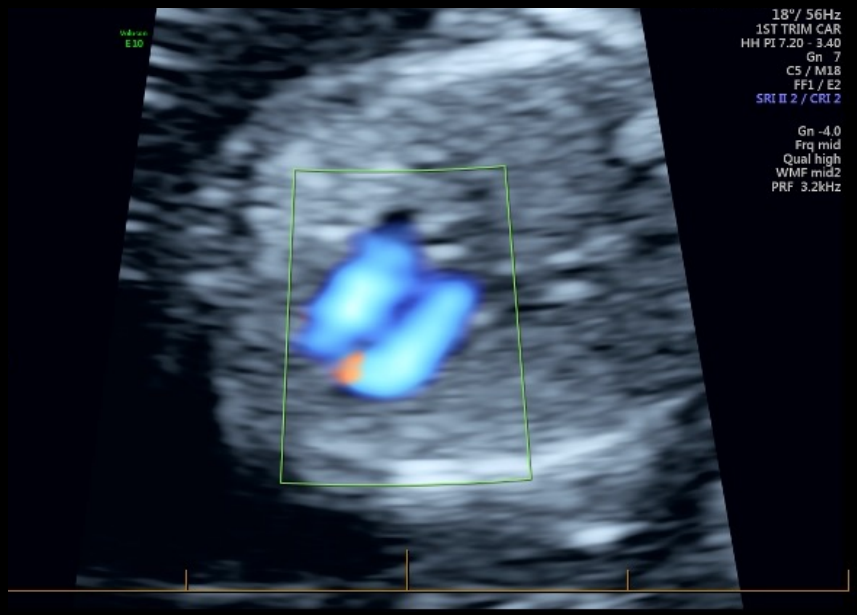
13 + 2 weeks

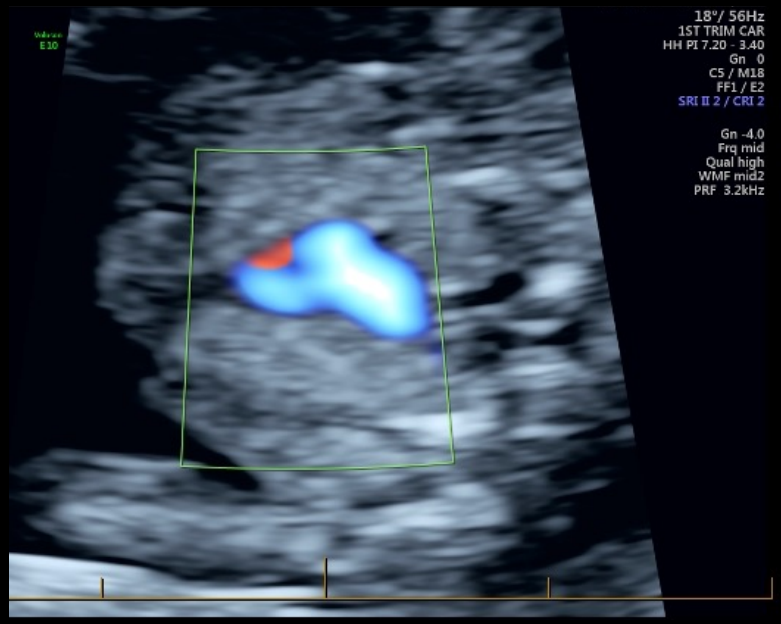

E10
42°/ 55Hz
Fet Cardio CRI
PI 14.10 - 4.30
Gn -2
C4 / M1
FF1 / E3
SRI II 2 / CRI 3

11 + 6 weeks

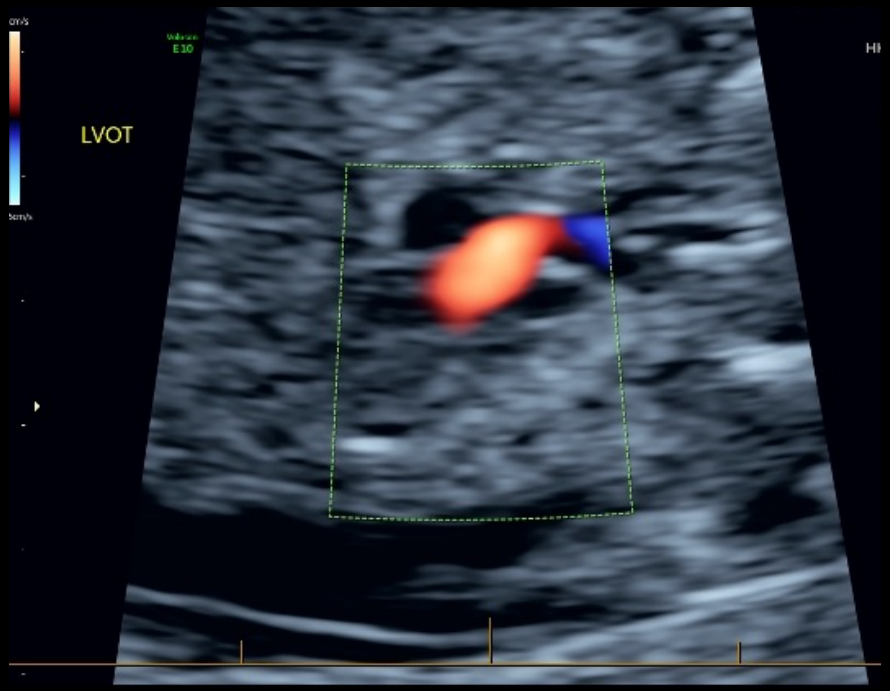

31.5
A Pure+ Precision+
4
5
6
7
10C3
22 fps
G:84
DR:65
DF 3.8
CG:35
6.2k
F:3
Precision+ A Pure+
24.7
4
5
6
7
10C3
diffII6.0
23 fps
G:84
DR:65
DF 3.8
CG:35
7.3k
F:3

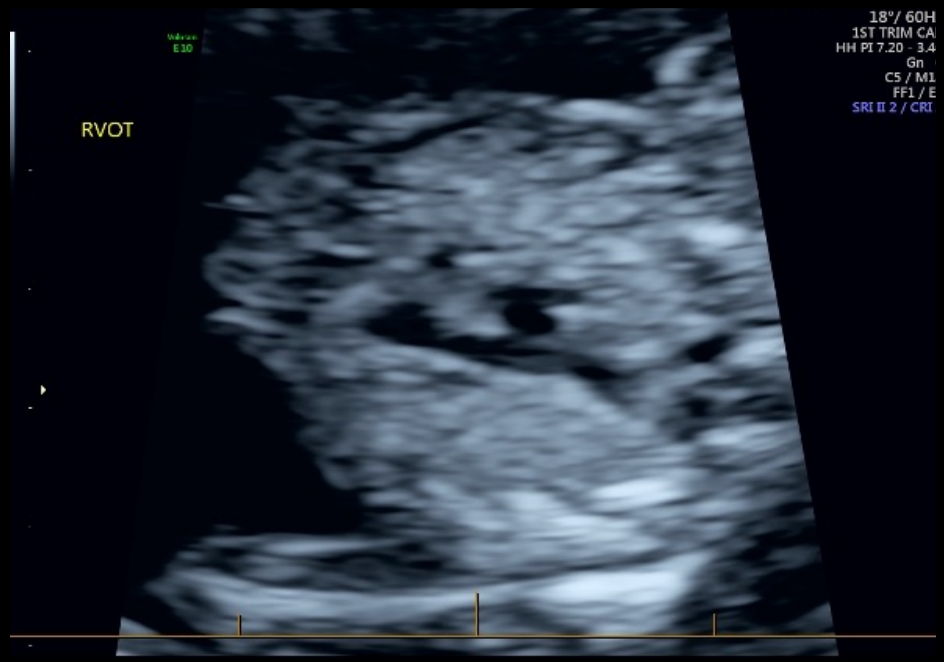
13 + 0 weeks


18°/ 56Hz
1ST TRIM CAR
HH PI 7.20 - 3.40
Gn 7
C5 / M18
FF1 / E2
SRI II 2 / CRI 2
Gn -4.0
Frq mid
Qual high
WMF mid2
PRF 3.2kHz

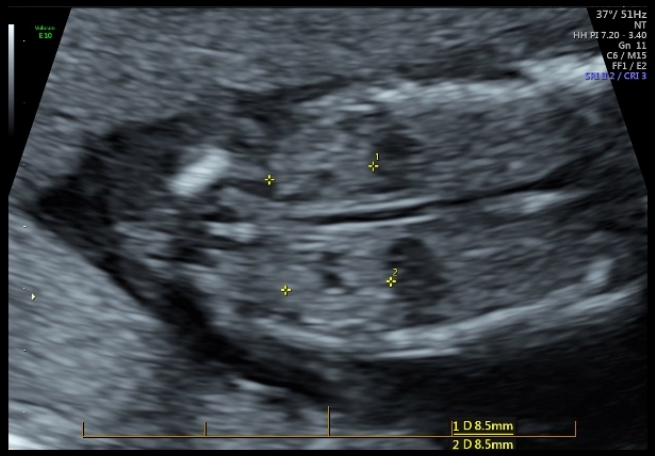

18°/ 56Hz
1ST TRIM CAR
HH PI 7.20 - 3.40
Gn 0
C5 / M18
FF1 / E2
SRI II 2 / CRI 2
Gn -4.0
Frq mid
Qual high
WMF mid2
PRF 3.2kHz

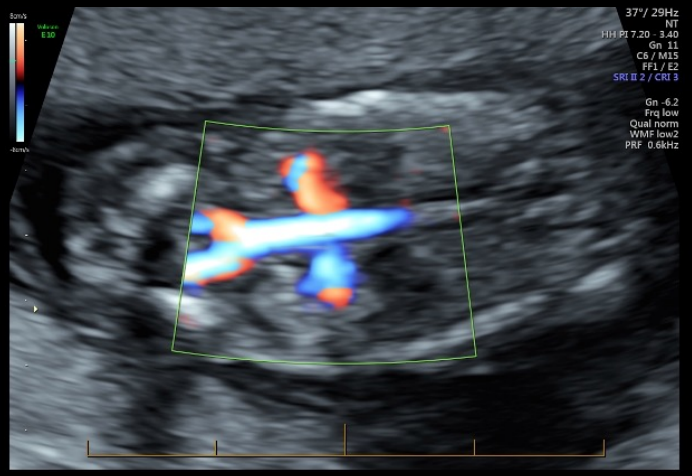
13 + 0 weeks



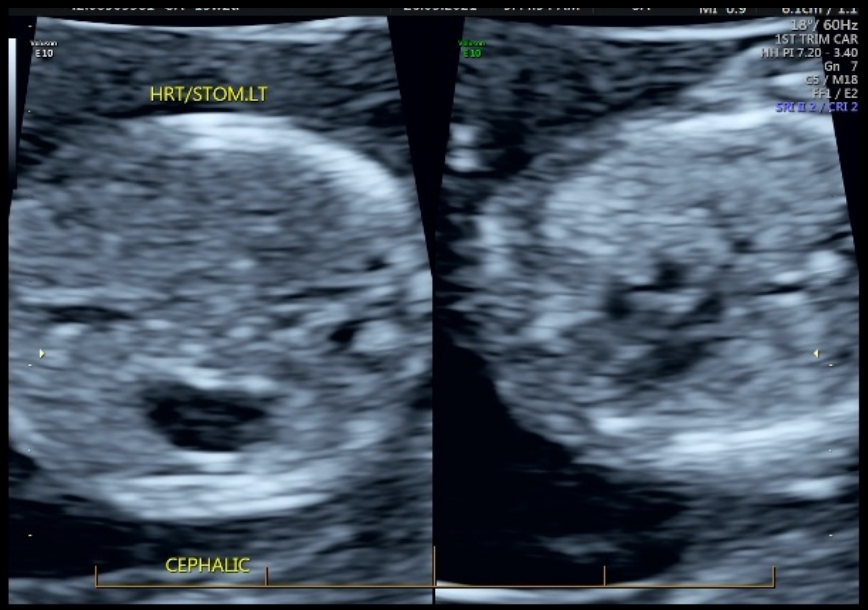


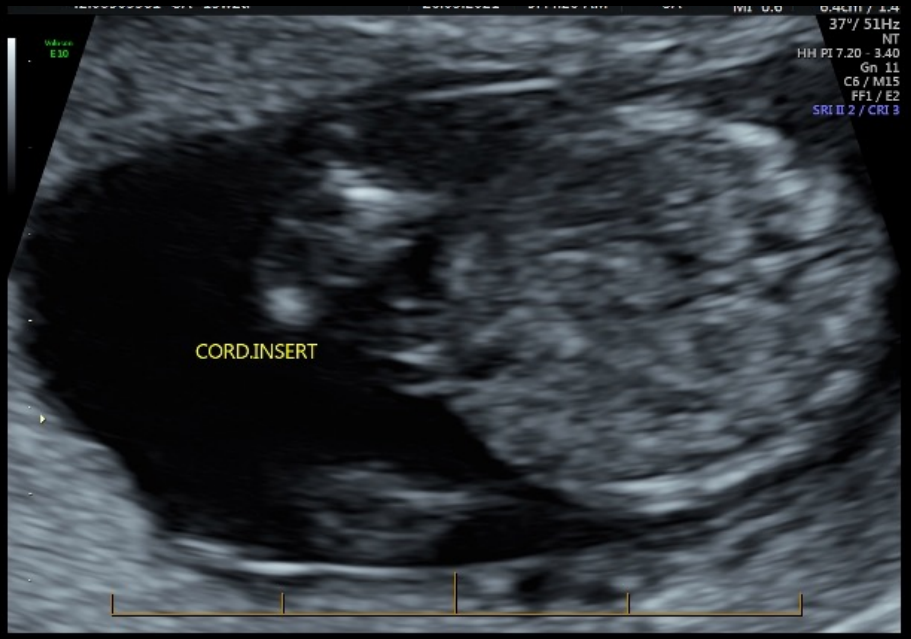
13 + 2 weeks


37°/ 51Hz
NT
HH PI 7.20 - 3.40
Gn 11
C6 / M15
FF1 / E2
1 D 8.5mm
2 D 8.5mm

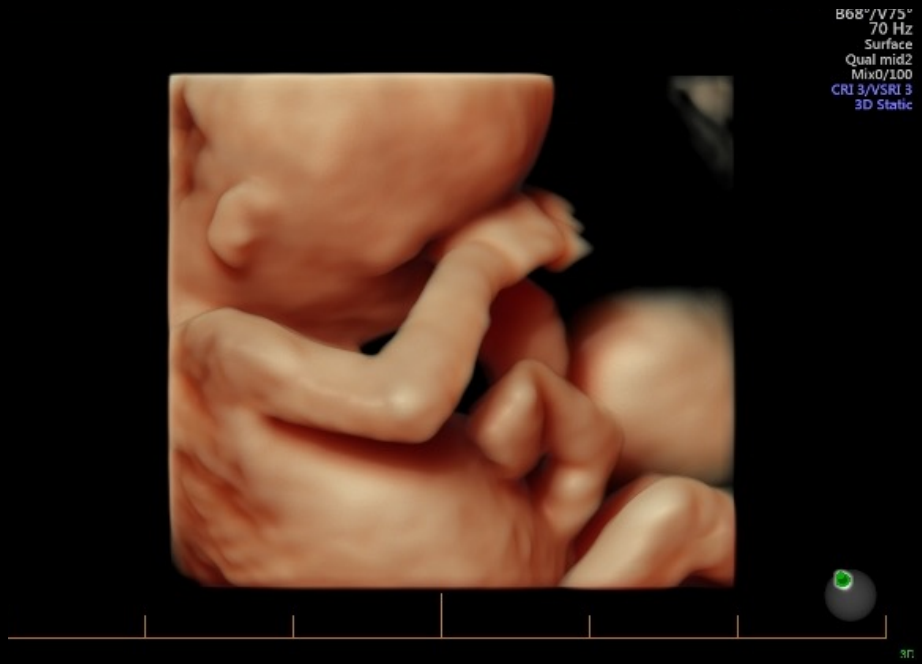

37°/ 29Hz
NT
HH PI 7.20 - 3.40
Gn 11
C6 / M15
FF1 / E2
Gn -6.2
Frq low
Qual norm
WMF low2
PRF 0.6kHz

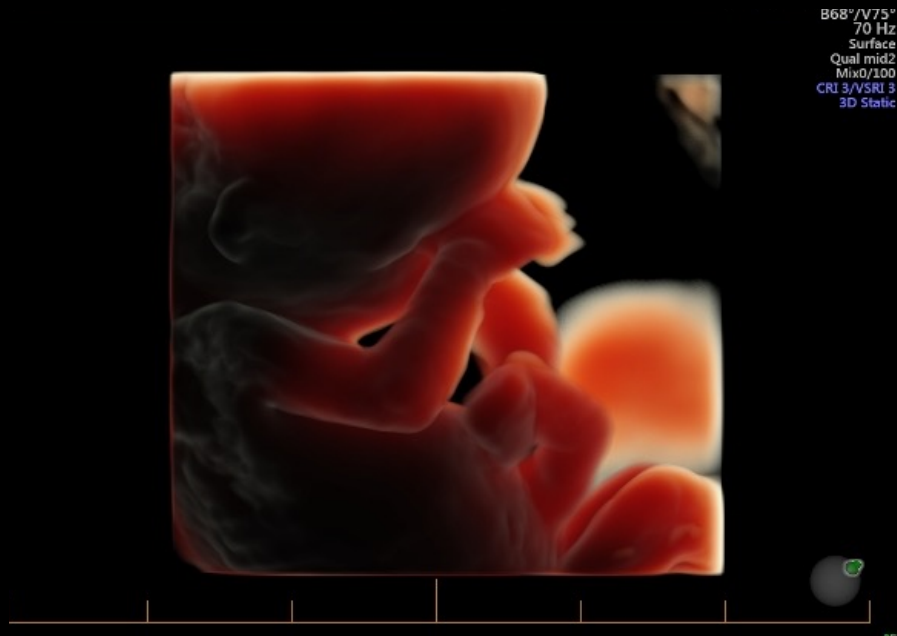
13 + 2 weeks





TV scanning


B68°/V75°
70 Hz
Surface
Qual mid2
Mix0/100
CRI 3/VSRI 3
3D Static


B68°/V75°
70 Hz
Surface
Qual mid2
Mix0/100
CRI 3/VSRI 3
3D Static

11-13 weeks anomaly detection

Syngelaki A et al, UOG, 2019;54:468

Detection %	Abnormality
100%	Gastroschisis, omphalocele, acrania, Body stalk anomaly, Alobar holoprosencephaly, encephalolcele,
>50%	Open NTD (59%), HLHS (92%), AVSD (91%), complex heart defect (60%), Absent extremities (75%), fetal akinesia (73%), lethal skeletal dysplasia (71%), Lower UT obstruction (71%)
<10%	Agenesis Corpus callosum, isolated cleft lip, CPAM, VSD, unilateral renal agenesis, abdominal cysts,

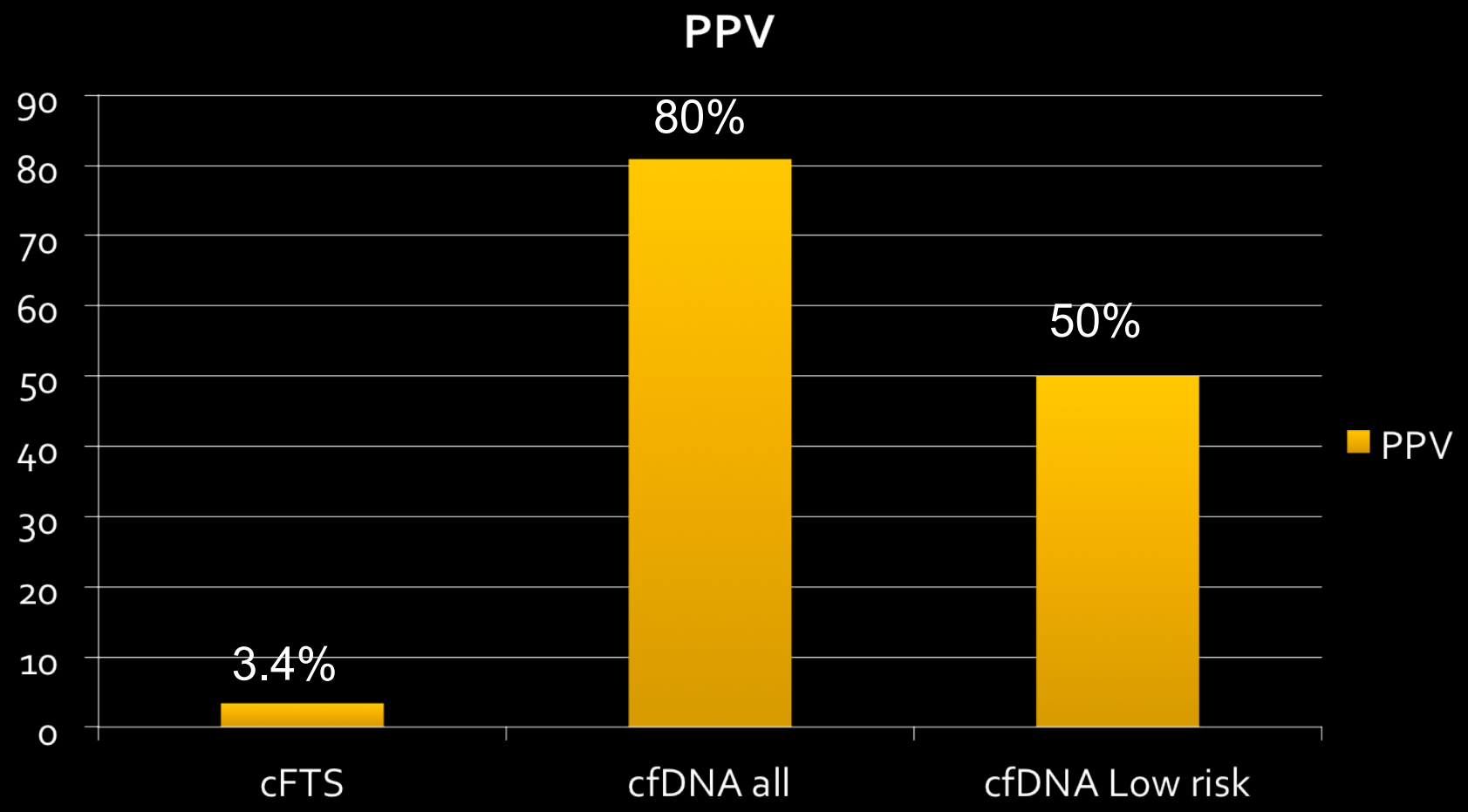
First Trimester Ultrasound

- **RANZCOG College Statement (2018):**
  - *"Women who choose to have cfDNA as a primary screening test should still be offered the opportunity to have an 11-13 week ultrasound for an early structural assessment, as 50% of major abnormalities can now be detected at this gestation".*

Question 4

- How should a “positive” NIPT be interpreted?

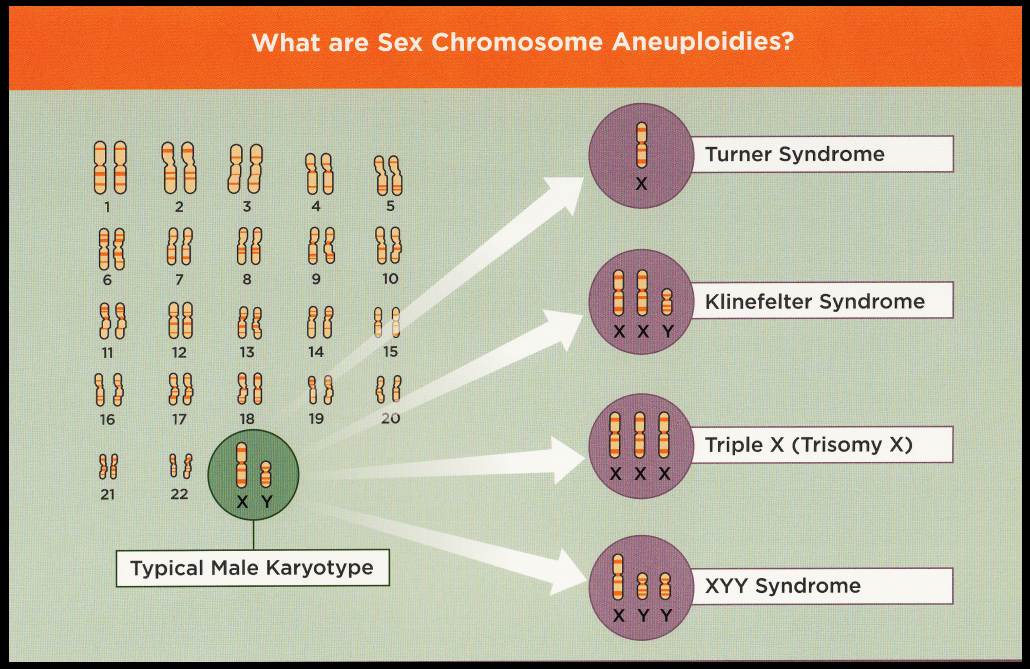
PPV: cFTSvs NIPT vs low risk NIPT



Norton ME et al NEJM, 2015

Question 5

- NIPT in sex chromosomal abnormalities (SCA)?
- To do or not to do?



Importance in screening!

- Clinically significant abnormality that has significant impact on development !

Sex Chromosome Abn (SCA) Counselling

- High prevalence
  - Potential High frequency of Positive NIPT
  - 1%
- Phenotypic features
  - Highly variable
- Relatively few serious physical abnormalities

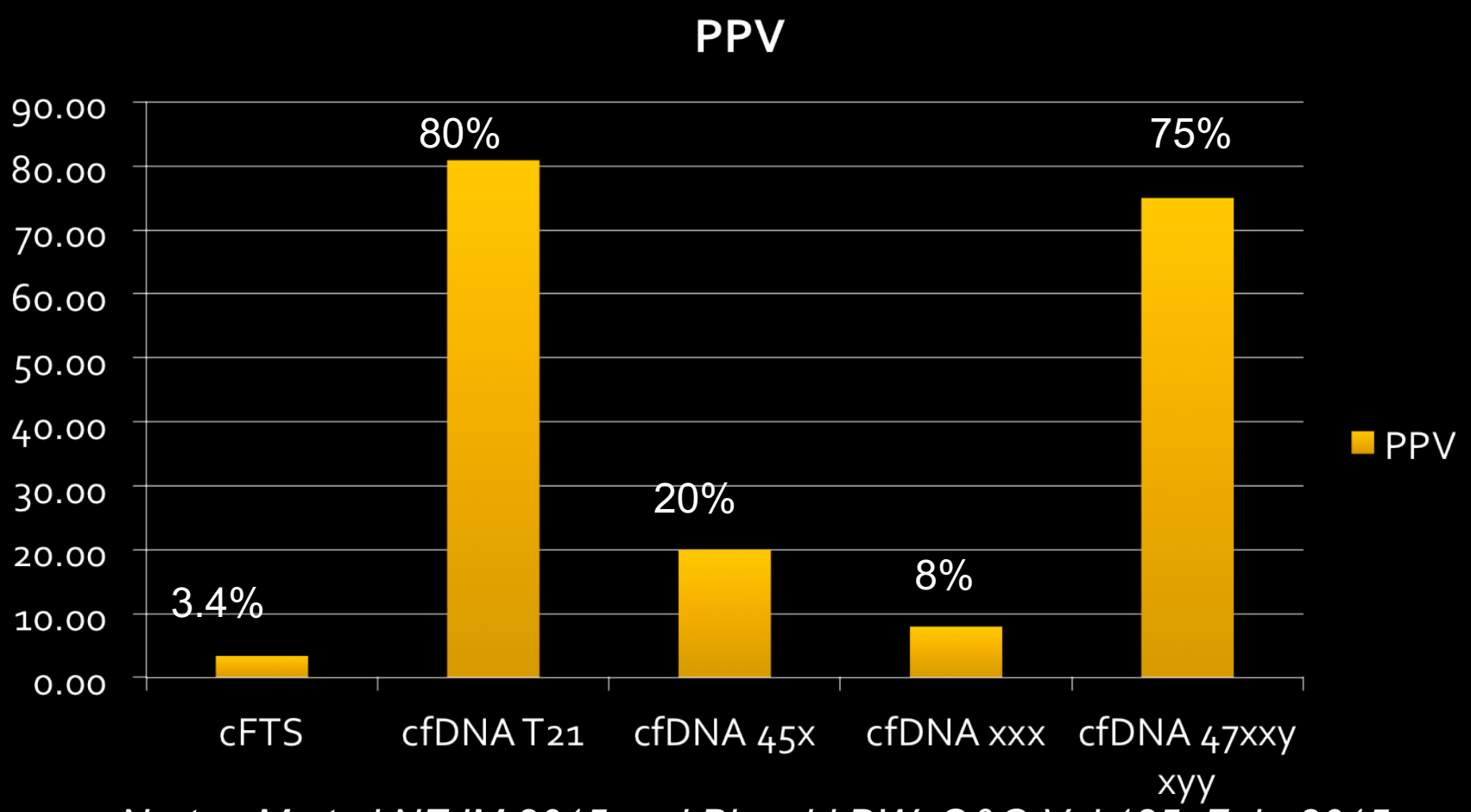
Mennuti MT et al Prenatal Diagnosis 2015,35,980-985

Sex Chromosome Abn (SCA) Counselling

- Phenotypic ascertainment bias
  - Postnatal/adult –
    - over-representation of severe clinical outcome
  - Prenatal detection
    - Better outcomes seen
- Benefit from early screening and medical intervention

Mennuti MT et al Prenatal Diagnosis 2015,35,980-985

PPV: SCA



Norton M et al NEJM 2015 and Bianchi DW, O&G, Vol 125, Feb. 2015

13,000 Aussie males don't know they have Klinefelter's Syndrome and remain untreated

Are you one of them?

Normal Adult

Abnormal Adult

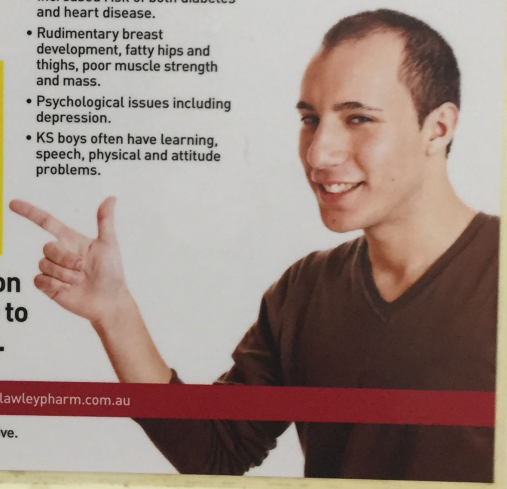
The physical signs of Klinefelter's Syndrome are:

- Under-functioning testicles that are hard and abnormally small – peanut size.
- infertility (recent technological advances can assist KS males to father a biological child)
- reduced life-span if untreated.
- Increased risk of both diabetes and heart disease.
- Rudimentary breast development, fatty hips and thighs, poor muscle strength and mass.
- Psychological issues including depression.
- KS boys often have learning, speech, physical and attitude problems.
- When diagnosed, treatment provides KS males with vastly improved mental and physical health and lifestyle opportunities.

Please consult your doctor.

check your balls .com.au

Self examination is the first step to your wellbeing.



LAWLEY www.lawleypharm.com.au

A Lawley men's health initiative.

However

- RCT 47XXy - Treating with lose testosterone age 4-12
- Positive effects on visual-motor integration and psychosocial function, without affecting most other motor or cognitive outcomes
- Positive effects on several aspects of anxiety/depression and social functioning, without adverse effects on behavior.

Ross JL et al J Pediatr 2017;185:193-9

Sex Chromosome Abn (SCA) Counselling

- Parental decision making
  - Significant decrease in proceeding GTOP with multidisciplinary counselling
  - Need to provide accurate, unbiased and updated information

Mennuti MT et al Prenatal Diagnosis 2015,35,980-985

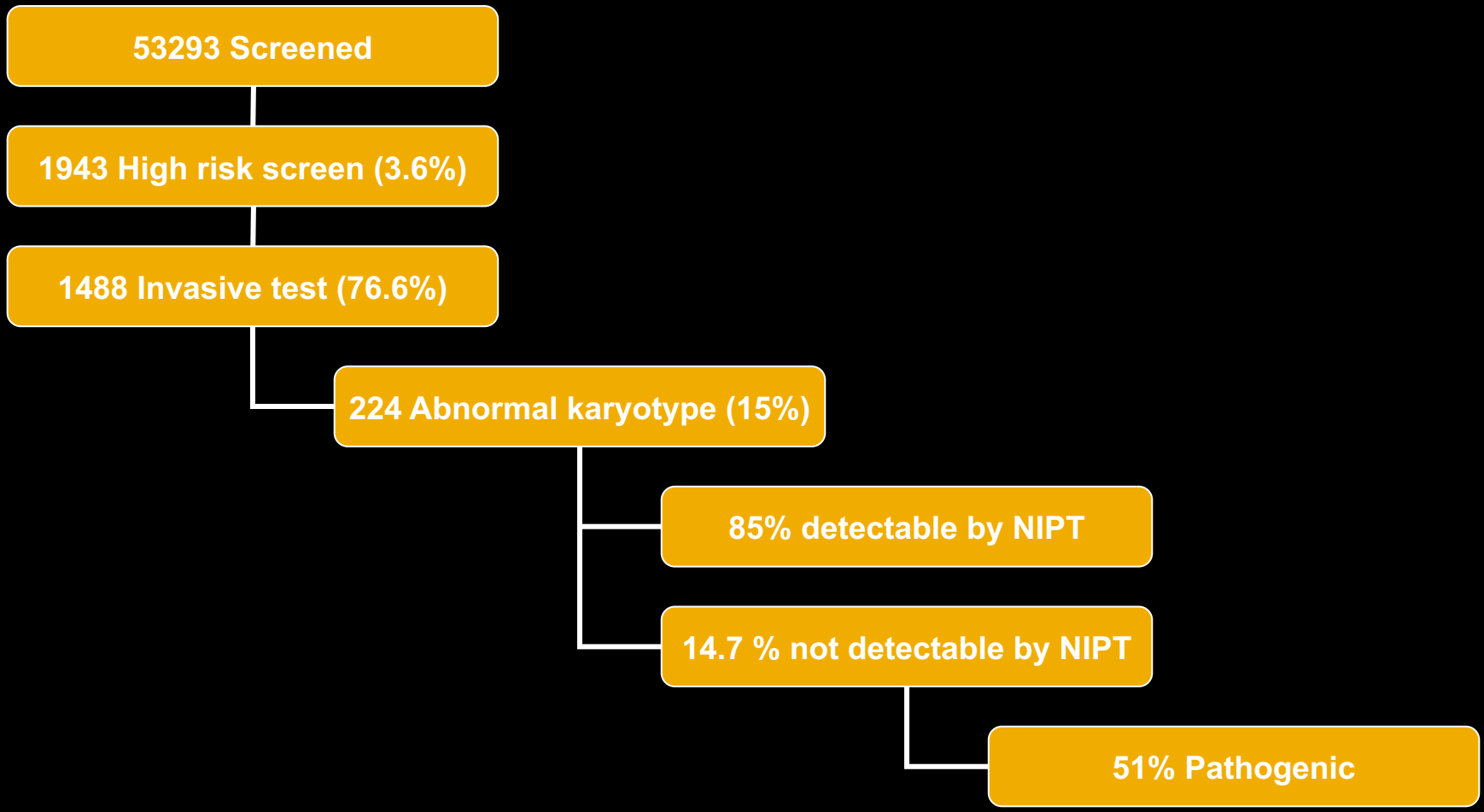
ISPD 2015

- *"Women should have the option to separately accept or reject the sex chromosome analysis"*

Question 7

- What is the chance of atypical aneuploidy occurring in high risk FTCS and “low risk” NIPT?
- How does ultrasound help?

Western Australia 2007-2009



Maxwell S et al, ANZJOG 2015;55:420-426

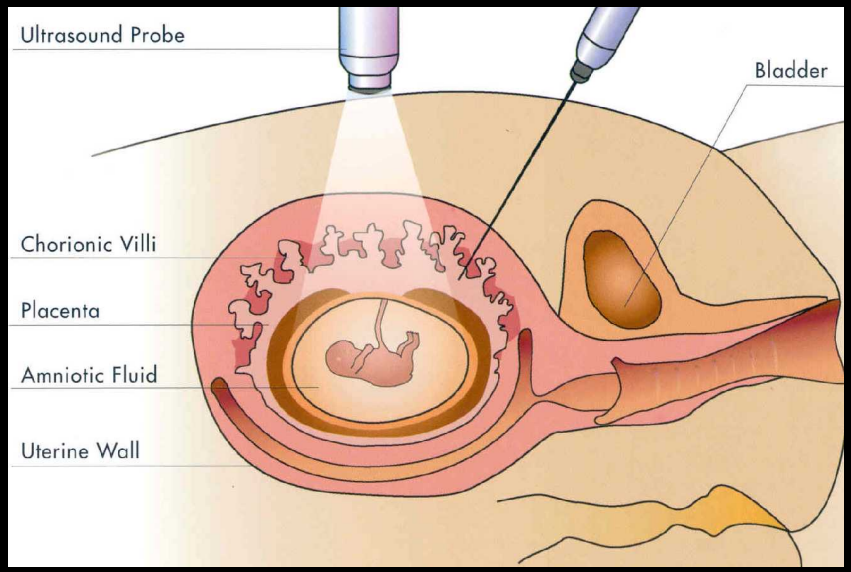
Western Australia- 2007-2009
53,000 women screened

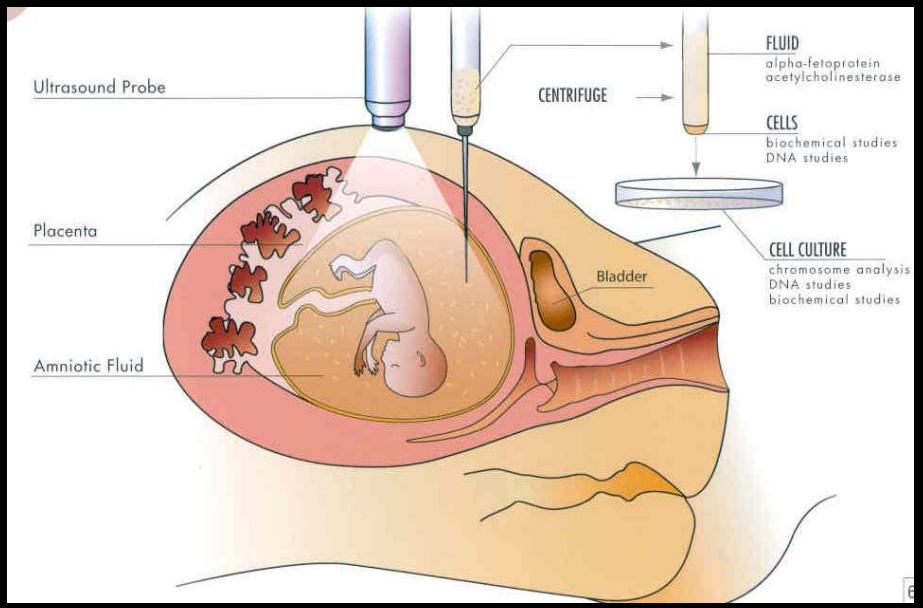
- *"Fetal Sonographic appearance was likely to have led to recommendation for invasive test"*
- *FTS risk <1:50 + Low risk NIPT + no ultrasound findings*
- *Residual risk of 0.33%*

Maxwell S et al, ANZJOG 2015;55:420-426

Question 9

- What really in the risk of miscarriage from invasive testing?





Procedure related loss meta-analysis

- Amniocentesis
  - << 1:300. ?1:909
- CVS
  - << 1:300 ?1:454

Akolekar R et al, UOG. 2015;55:541-6

Counseling

- "Best case scenario"
  - ***Rieder W et al ANZJOG. 2018. 58:397-403***
- Medical legal risk to *over estimate risk* and miss atypical chromosomal abnormalities
- Optimal choice based on experienced operator
  - ISPD Newsletter Vol 1, Number 1, December 2012

Question 11

What is considered an elevated NT?

Specialized morphology?

Risk of selected structural abnormalities in infants after increased nuchal translucency measurement

Rebecca J. Baer, MPH; Mary E. Norton, MD; Gary M. Shaw, DrPH; Monica C. Flessel, PhD; Sara Goldman, MPH; Robert J. Currier, PhD; Laura L. Jelliffe-Pawlowski, PhD

Background risk of major birth defects = 2%

NT	<90th%	90-94th%	95th-99th% 2.7-3.4	99th% >= 3.5mm
Structural Abnl	1.7%	2.1%	2.7%	5.2%

Baer RJ et al AJOG Dec 2014

NIPT or Invasive testing for all > 3.5 mm?

- Isolated NT > =3.5 mm
  Incremental yield of 4.0% to standard karyotype
- Significant limitations for NIPT
- We would offer invasive diagnostic testing.

Grande M et al UOG 2015: 46: 650-58.

NT 3.0 to 3.4

NT	Microarray Abnomrlity	Standard NIPT LR	Genome Wide NIPT
Background	1%		
NT < 3.0 mm	0.8%		
NT 3.0-3.4 mm	4.7%	1.9%	1.5%

Sagi-Dain L et al O&G, VOL. 137, NO. 1, JANUARY 2021

Increased NT > 1.9 MoM

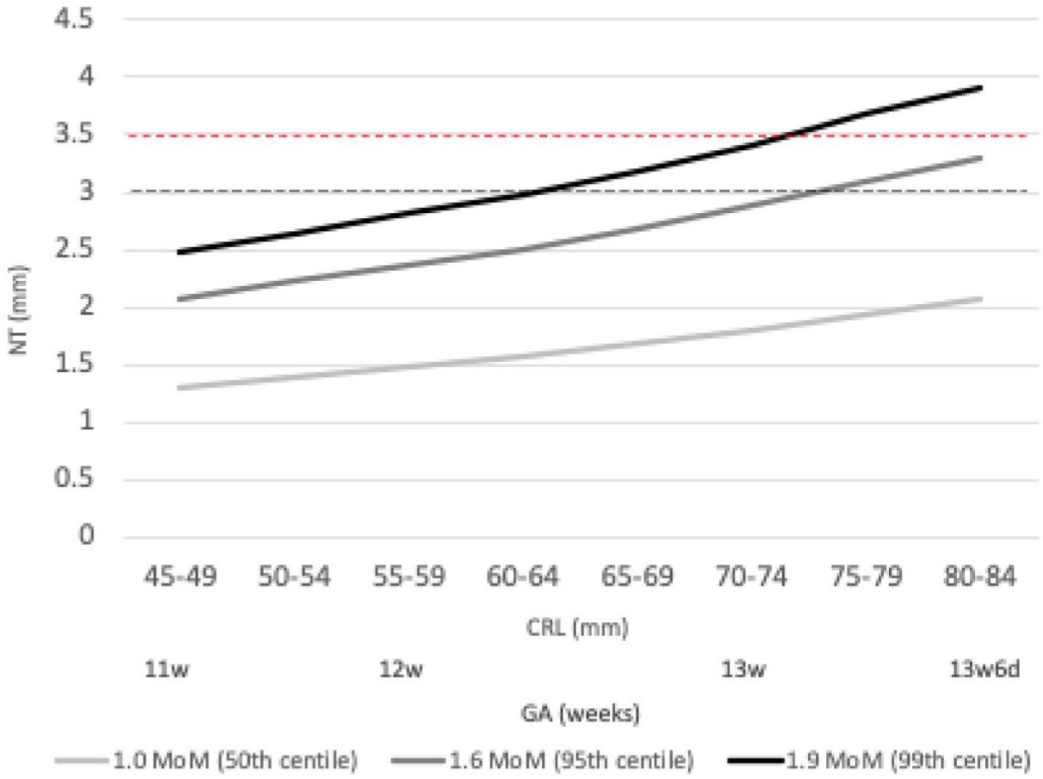
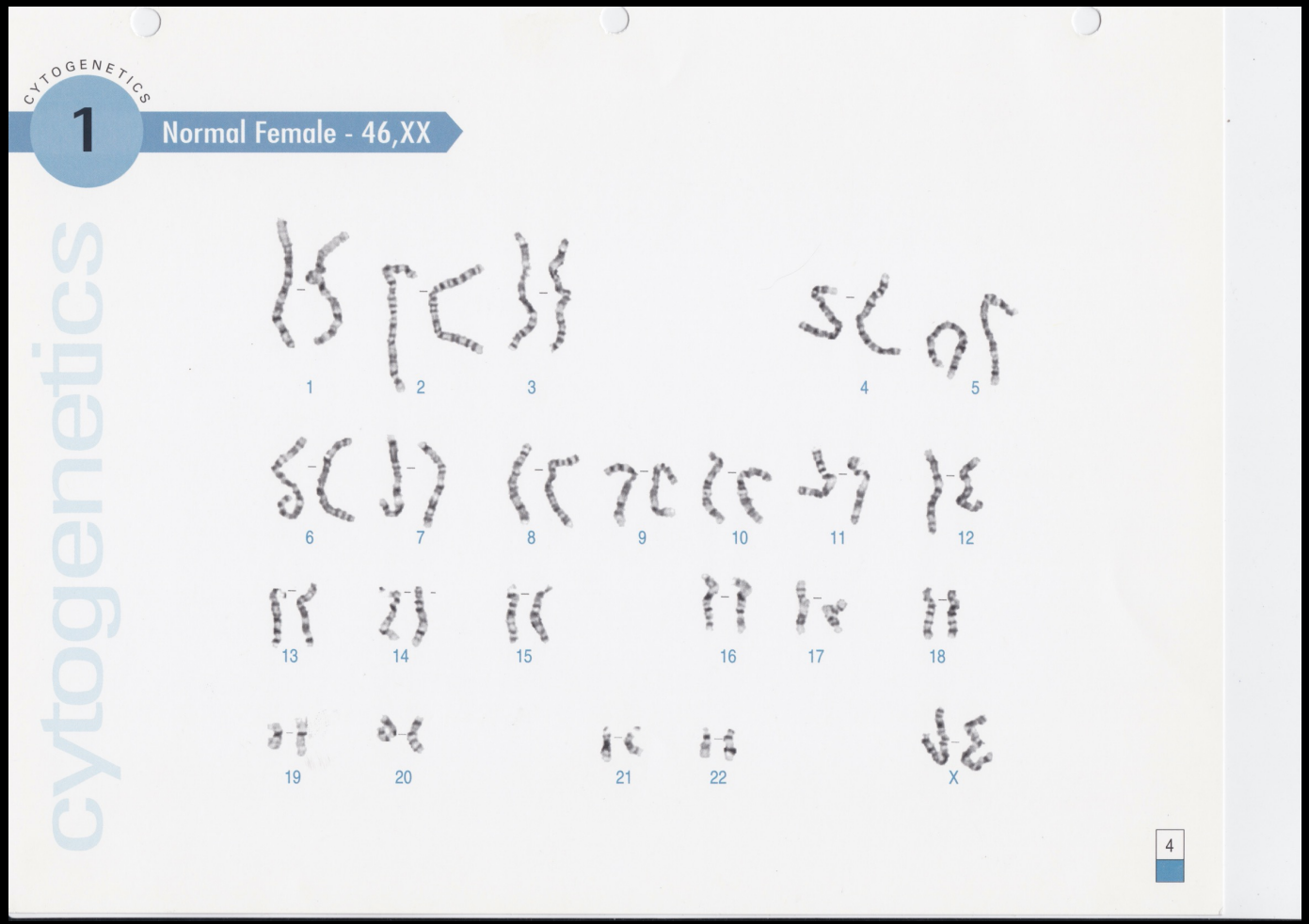


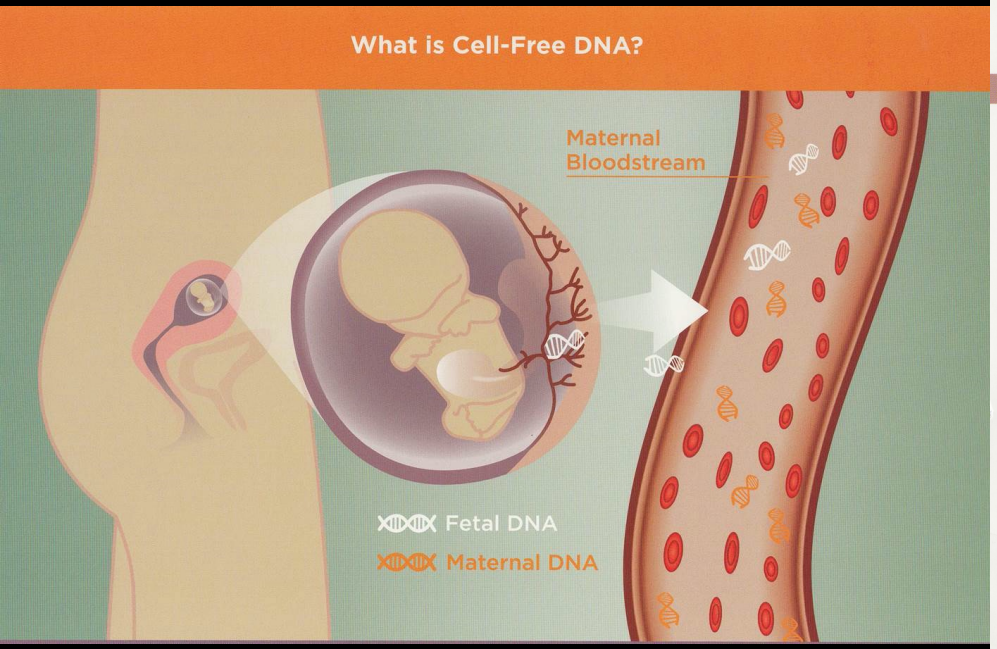
FIGURE 2 Distribution of nuchal translucency measurements among 81,244 singleton pregnancies in Victoria, 2015–2016. CRL, crown rump length; NT, nuchal translucency, MoM, multiples of the median. *Source:* Data courtesy of Leonard Bonacquisto, Victorian Clinical Genetics Services. Software: Alpha Version 8.0.16281.67, Logical Medical Systems Ltd, London, United Kingdom [Colour figure can be viewed at wileyonlinelibrary.com]

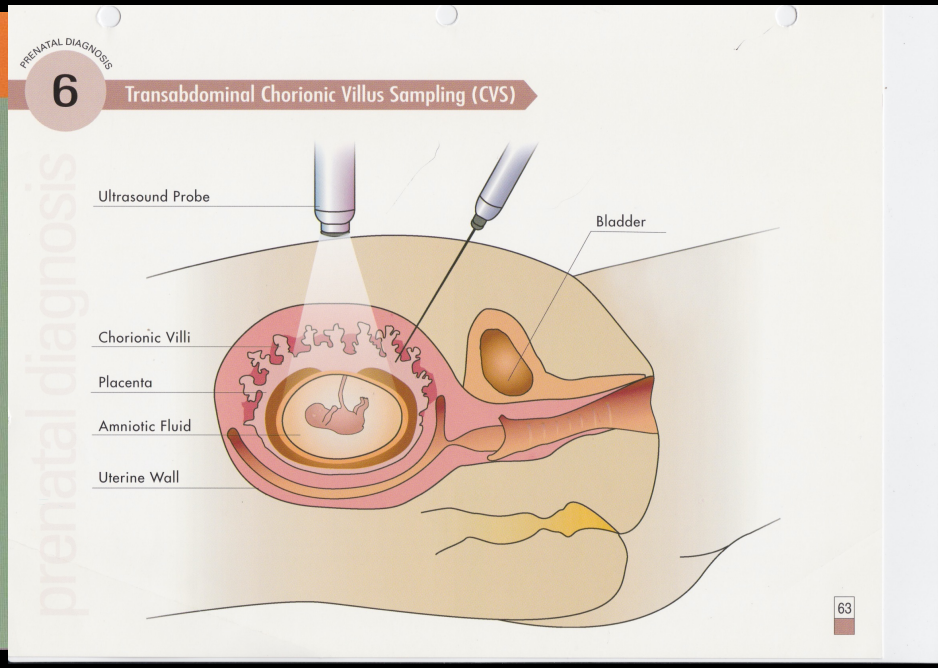
Kelley J et al Prenatal Diagnosis. 2021;41:1305–1315.

Question Genome Wide NIPT
Should we tick the box?

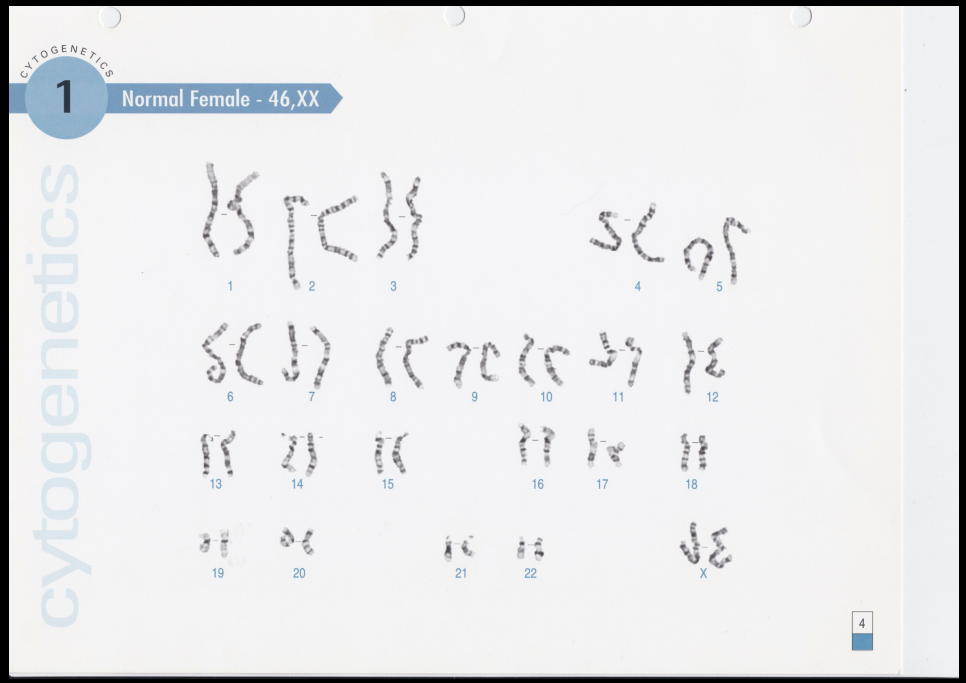


Where does cfDNA come from?



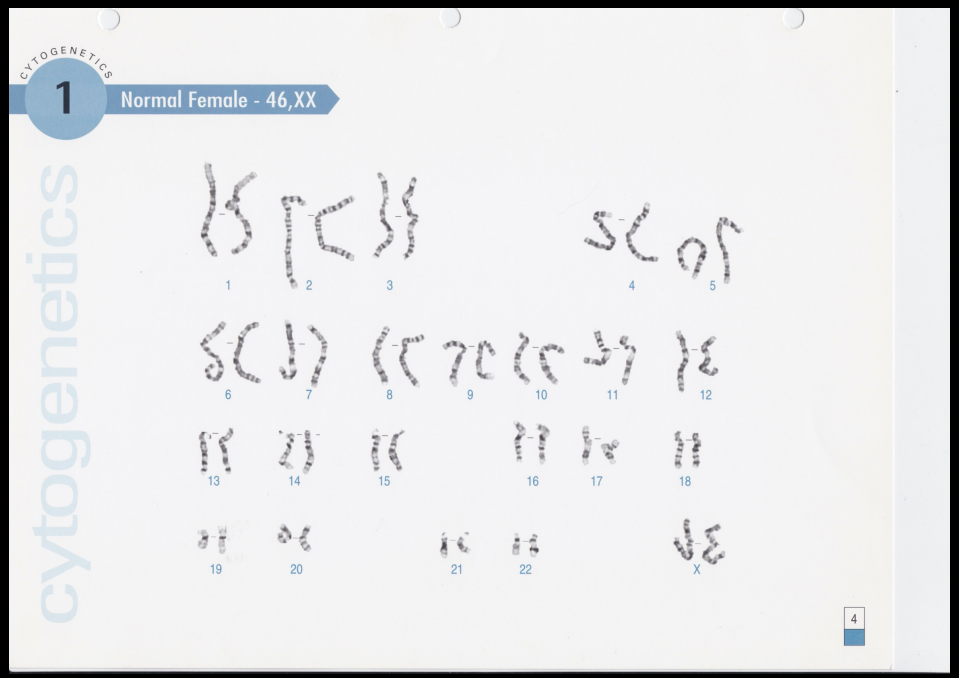


Question Genome Wide NIPT Should we tick the box?



- Common (T21, T18, T13) which account for over 70% of chromosomal abnormalities seen standard karyotype
- There is high level evidence for its use.
- Genome Wide NIPT
  - Clinpath
  - Repromed

Question Genome Wide NIPT Should we tick the box?



- Rare Autosomal Trisomies (RATs) or large segmental chromosomal abnormalities
  - 1:250 to 1:300
  - So common
- 7.5 Mb size
- Double your high risk NIPT result

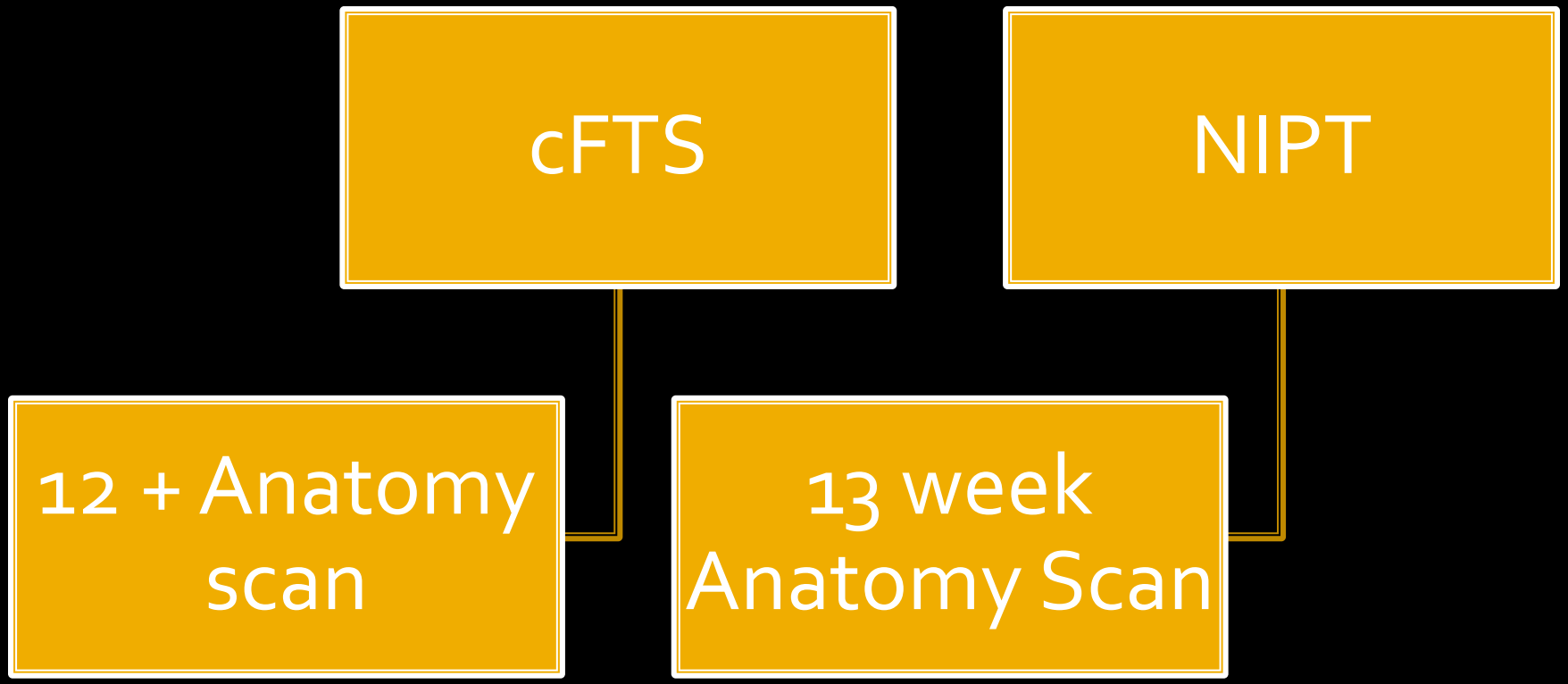
Genome Wide NIPT

- True complete fetal trisomies (other than T21, T18 or T13) are likely incompatible with normal pregnancy progression.
- A NIPT screen positive for RAT's, a normal ultrasound will indicate confined placental mosaicism (CPM) in 97% of the time.
- Fetal Mosaisim is rare 1.5% and ultrasound may be normal in 29% of cases
- CPM is a risk factor for fetal growth restriction, particular CPM of Trisomy 16.
- Approximately one in three NIPT high risk RAT will develop fetal growth restriction.

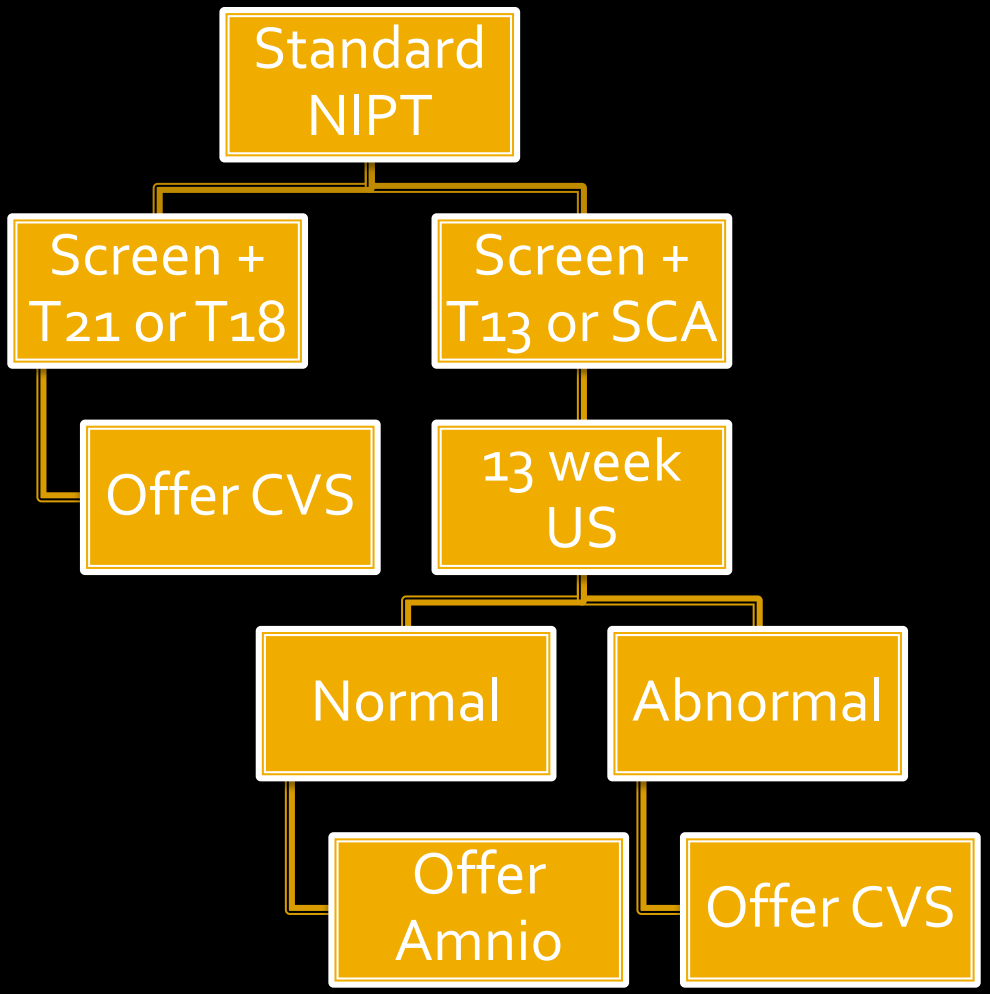
Genome Wide NIPT

- Suggest
  - Pretest counselling
  - For positive NIPT for RATs, the current recommendation is to first perform high level 12-13 week ultrasound
  - If the ultrasound is normal, since CPM will be the most common reason for the NIPT result, we recommend amniocentesis at 15 + weeks.
  - This is because NIPT is testing the same area that tested with CVS, placental DNA.

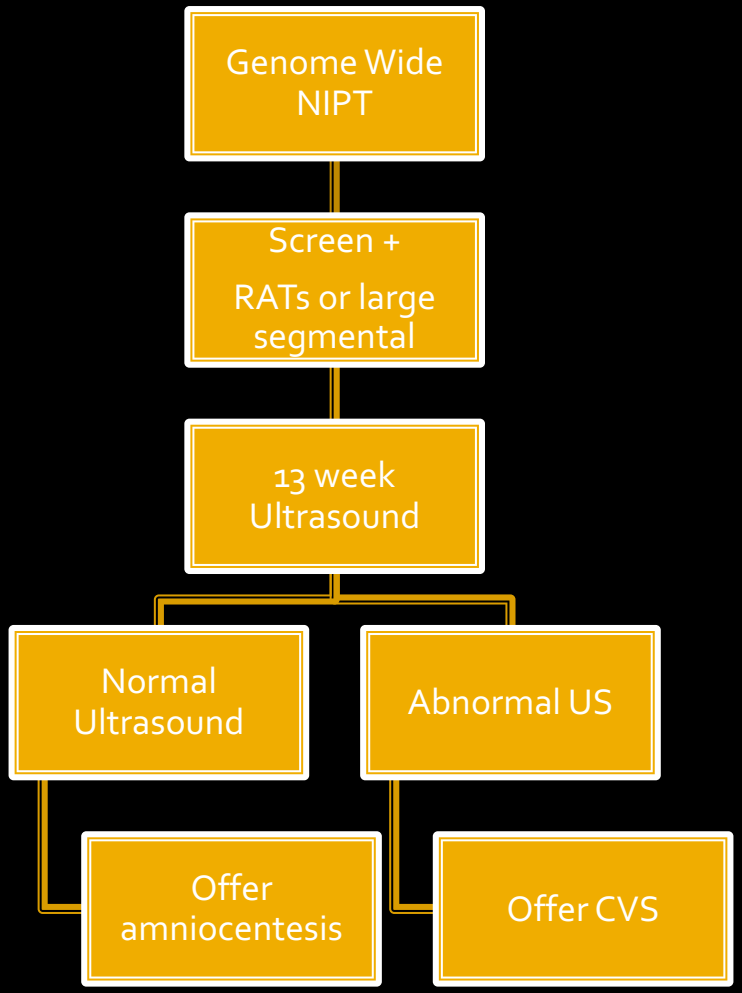
Recommendations



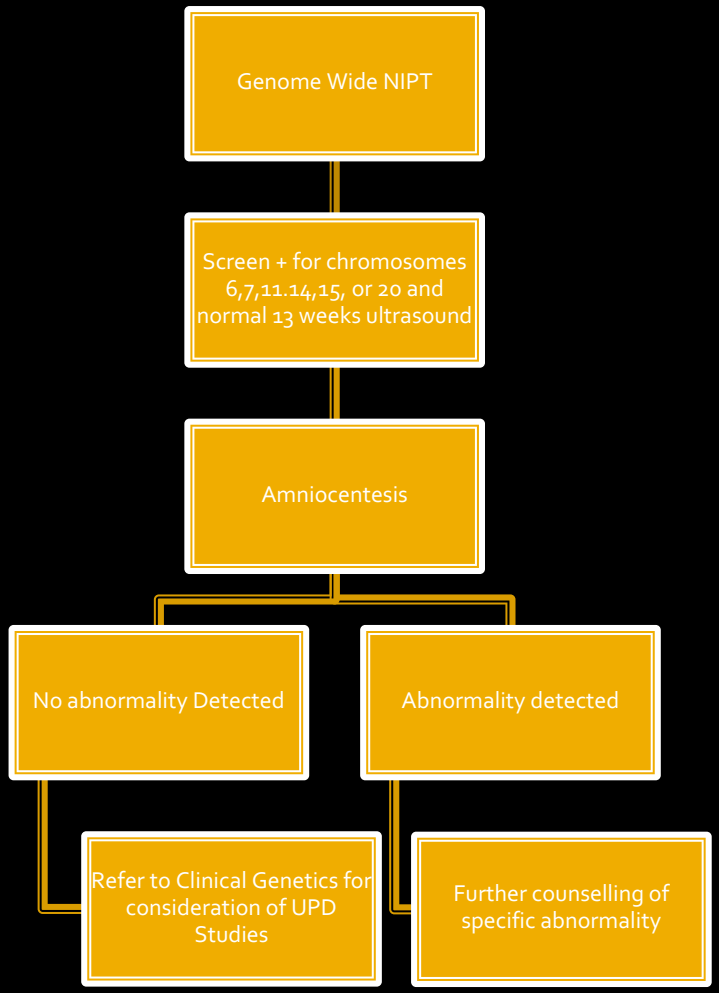
Recommendations



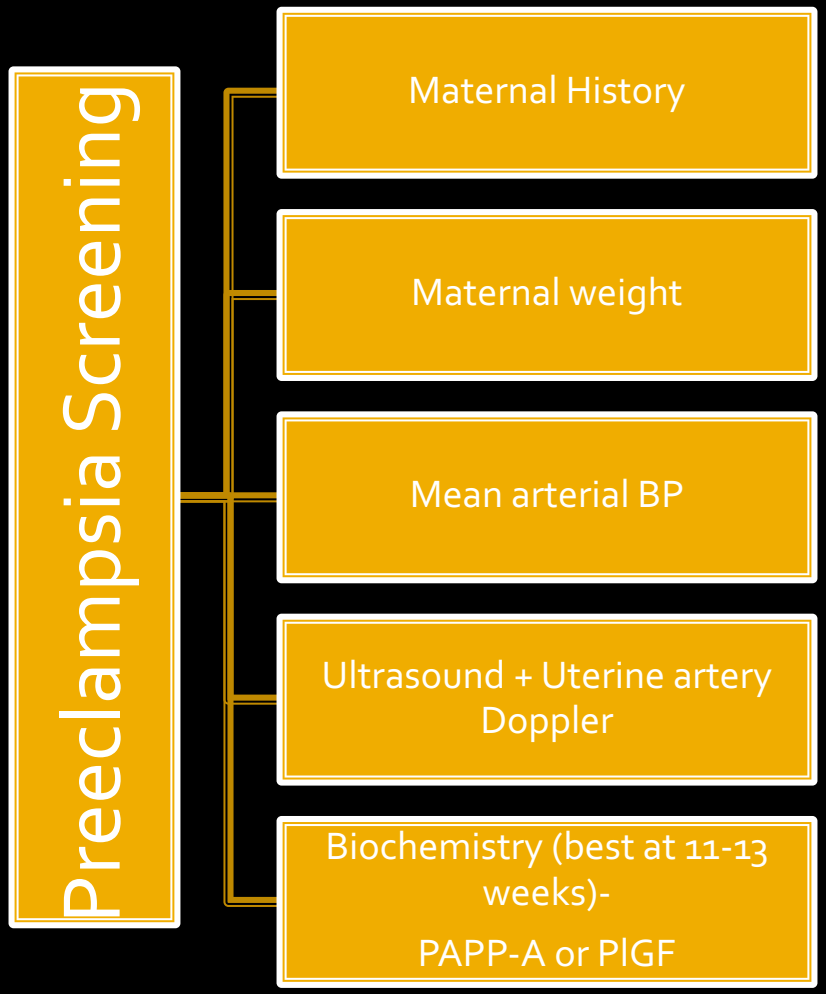
Recommendations



Recommendations



Preeclampsia Screening
Another talk



Thank You