

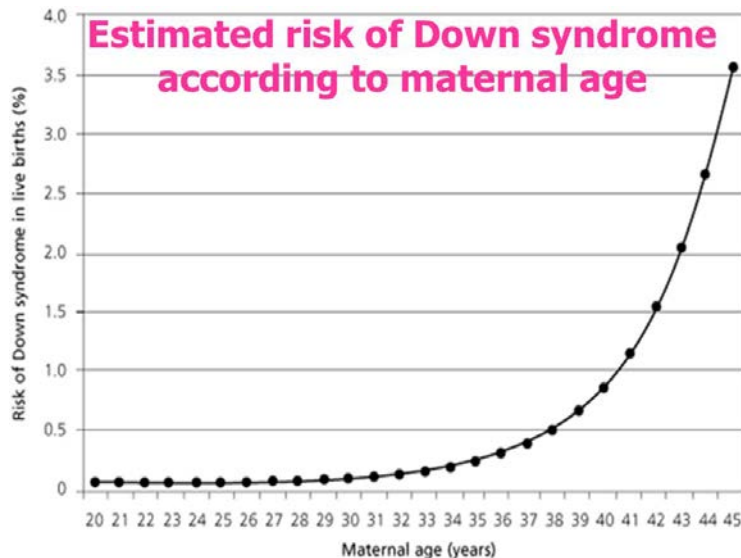
Module 7: Delving deeper

Module 7 will cover:

- *A Priori* risk
- Twins
- The false positive result
 - Mosaicism
 - Maternal chromosomal anomalies
 - Cancer
- The false negative result
 - When can a false negative result occur
 - Case studies
- Ultrasound anomalies
 - Structural anomalies
 - Sex aneuploidy
 - Microdeletions/
Microduplications
- Fetal Sex determination

A *priori* risk (background risk)

This is the starting or background risk for a person having a screening test. Some cell-free DNA tests use the maternal age-related statistics, at gestation as the '*a priori* risk'



- Most NIPT tests report a result without reflecting the individual's 'patient-specific' chance for any given trisomy (i.e. from quadruple or combined testing).
- In these cases, the use of the combined or the quadruple test results is a more appropriate approach when reporting on a high chance patient.
- The SAFE test is able to use the first trimester combined test result

SAFE test incorporates the first trimester combined test result (FTCT)

- The SAFE test has the option to include the use of First Trimester Combined Test (FTCT) for risk calculation
- If the FTCT is unavailable, the default prior is the maternal age
- This gives the most accurate, personalised and comprehensive prenatal screening result for pregnant women



SAFE screening report

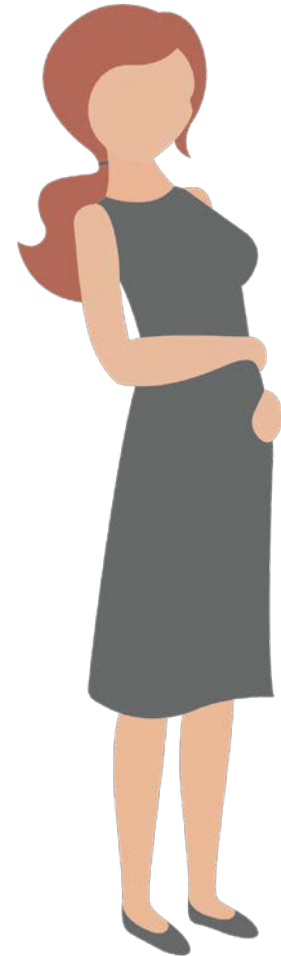
Trisomy	Background Risk	The SAFE test RISK SCORE	CLINICAL SUMMARY
Trisomy 21	1 in 171 ^{CT}	>95%	HIGH CHANCE INVASIVE TEST RECOMMENDED
Trisomy 18	1 in 542	Less than 1 in 1,000,000 (<0.0001%)	LOW CHANCE
Trisomy 13	1 in 1655	Less than 1 in 1,000,000 (<0.0001%)	LOW CHANCE

CT: Combined Test

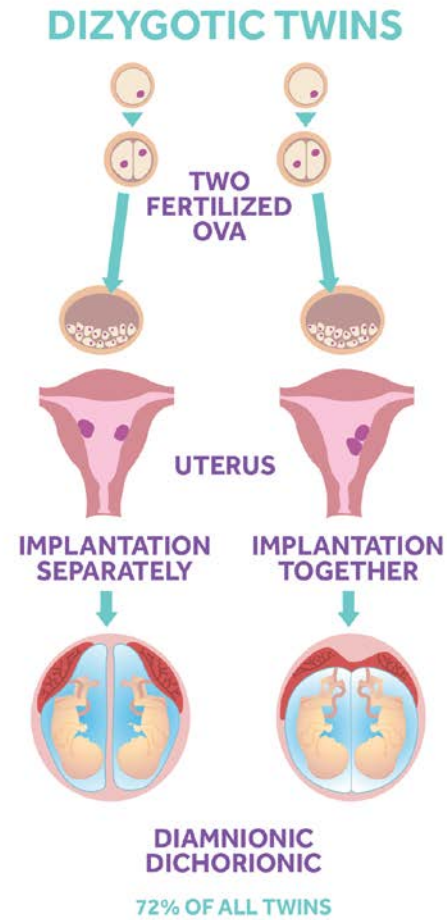
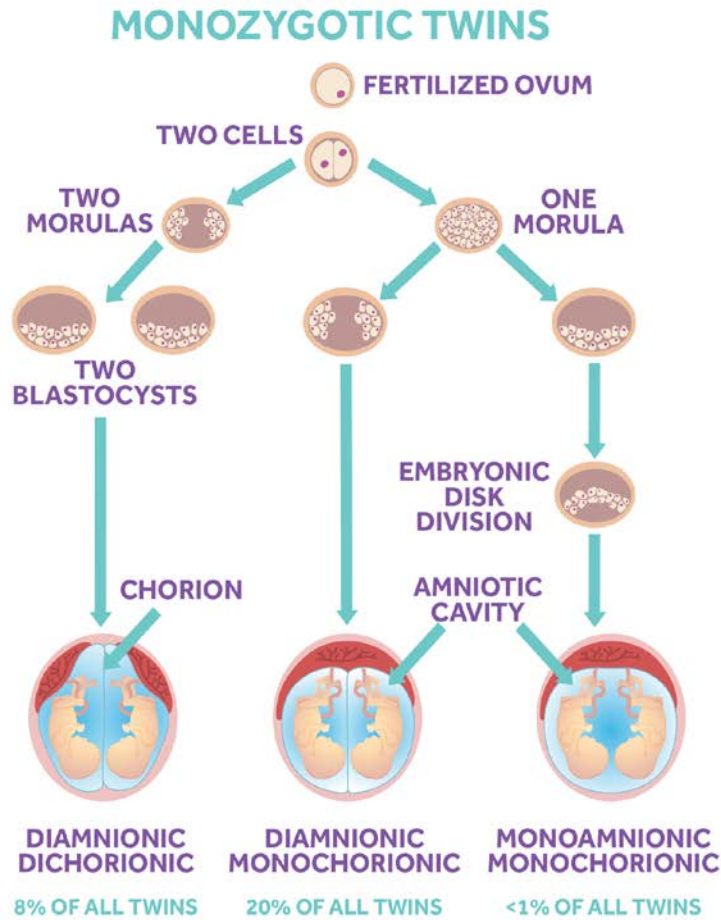
The sequencing data is automatically coupled with the background chance (FTCT or maternal age) to give an adjusted probability (risk score).

A priori risk case study

- A patient that is 21 years old would have a maternal age-related chance of 1 in 1450.
- When the sample is tested, the cfDNA result may halve the chance, giving a result of 1 in 2900 (low chance).
- If the same patient had the first trimester combined test (FTCT) first, and the result was 1 in 5; the overall result from the cfDNA test would be 1 in 10.
- The patient would remain high-chance and a diagnostic test would be offered.



Twin pregnancies



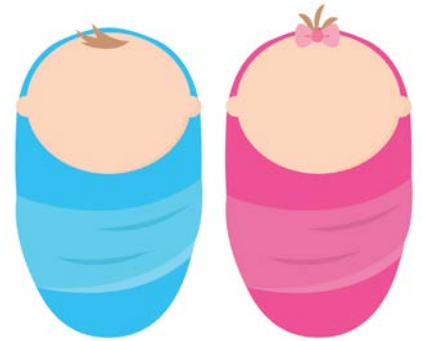
NIPT in twins



- Routine screening in twins has a higher false positive rate (~10%), with a lower detection rate than in singleton pregnancies.
- The most up-to-date systematic review of cfDNA screening in twin pregnancies reported – albeit on limited data – a 95% sensitivity and specificity for cfDNA screening in twins (Gil et al. 2017).
- In twin pregnancies, the cfDNA contribution of the two fetuses can vary significantly.
- It is possible that the fetal fraction of the affected fetus is below the threshold required for successful analysis, but there is a high contribution from the unaffected twin.

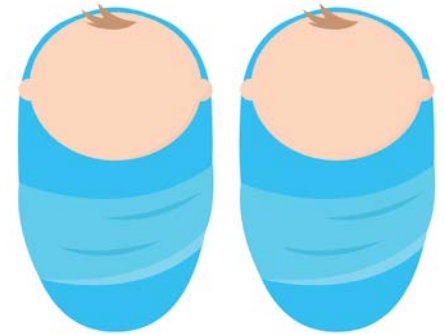
NIPT in dizygotic twin pregnancies

- There is a greater likelihood for Down's syndrome in most (dizygotic) twin pregnancies.
- Trisomy in dizygotic (non-identical) twins, only usually affects one twin. The contribution of each twin's cfDNA into the maternal blood may vary.
- In dichorionic twins, or if the chorionicity is unknown, the SAFE test software assumes they are heterozygous and modifies the fetal fraction requirement accordingly.
- Fetal sex determination is not available for dizygotic twins.



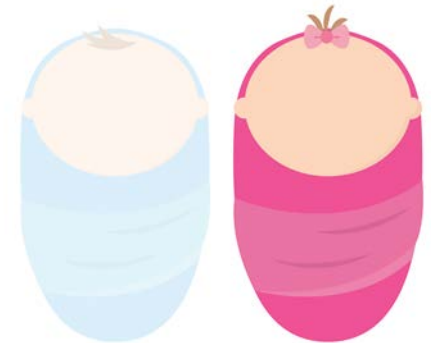
NIPT in monozygotic twin pregnancies

- The SAFE test is suitable for twin pregnancies. However, a high chance trisomy NIPT result in twins cannot determine which twin is affected.
- Performance of cfDNA screening in monozygotic (identical) twins is the same as for singleton pregnancies.
- Fetal sex determination is only available for singleton or monochorionic pregnancies, not dichorionic pregnancies.



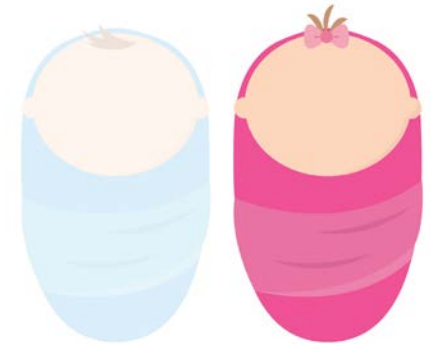
What is a vanishing twin?

- A vanishing twin, is a fetus in a multi-gestation pregnancy which spontaneously dies in the uterus during early pregnancy.
- It is then partially or completely reabsorbed by the other twin. In some instances, the dead twin will be compressed into a flattened, parchment-like state. This is also known as fetal resorption.
- Vanishing twins occur in up to one out of every eight multi-fetus pregnancies and may not even become known in most cases.



Vanishing twins and NIPT

- Early miscarriages are common when there is a chromosomal anomaly. It has therefore been suggested that vanishing twins may predispose to a high-chance result from a cfDNA screening test.
- There is no data to indicate how long the cfDNA from the demised twin remains in the circulation, but studies have suggested that 8 weeks from demise to testing would be a safe period, but often the date of demise is not known.
- If a vanishing twin is diagnosed, the mother should be advised that cfDNA performance is similar to that in dichorionic twin pregnancy.
- It is important to note on the patient consent form if a vanishing twin is found.



False positive result

Sometimes a cfDNA test result indicates a pregnancy is high chance for a trisomy. But it is then identified, either via an invasive diagnostic test or birth outcome, that the baby is unaffected. This is termed a false positive result.

What can cause a False Positive Result?

Conditions affecting the placenta:

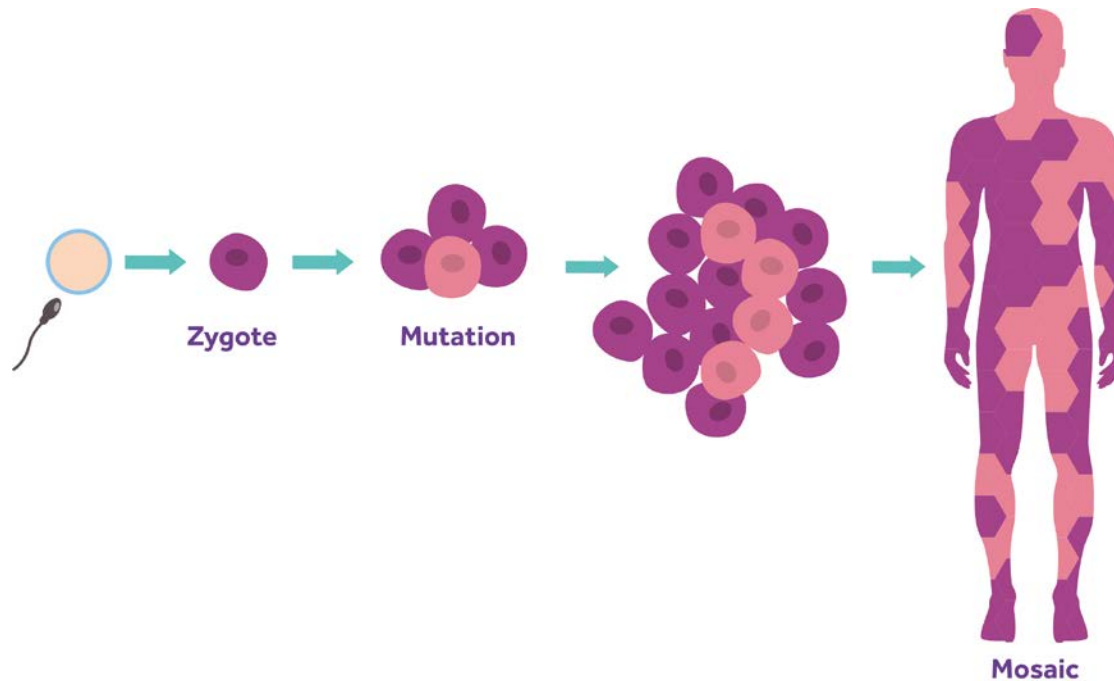
- Vanishing twin
- Placental mosaicism

Conditions affecting the mother:

- Partial duplication of a region of chromosome 21/18/13
- Maternal mosaicism for trisomy 21/18/13
- Cancer

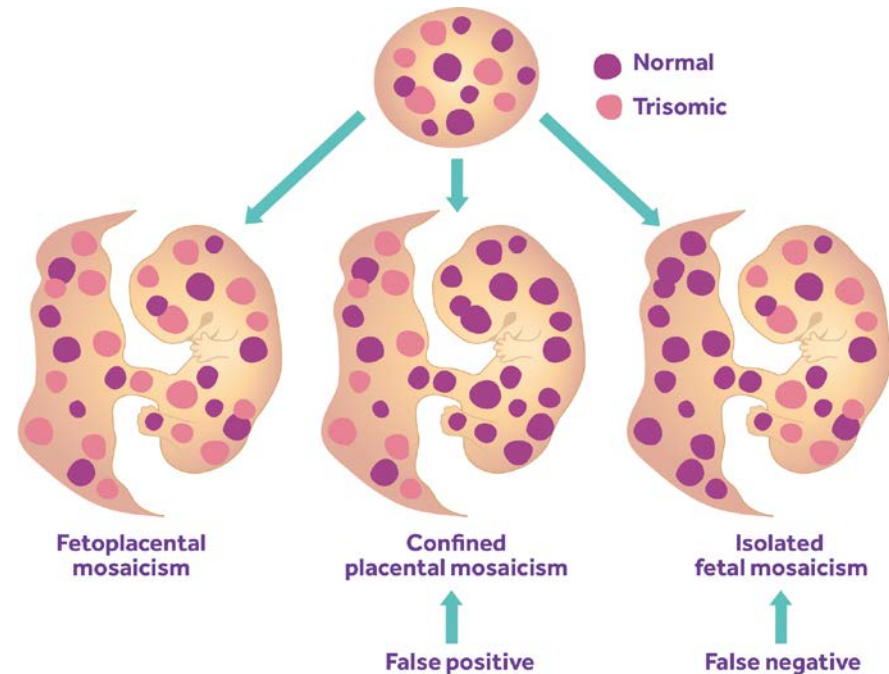
Mosaicism

When the genetic composition of an individual or organ is derived from two or more populations of cells with distinct karyotypes.



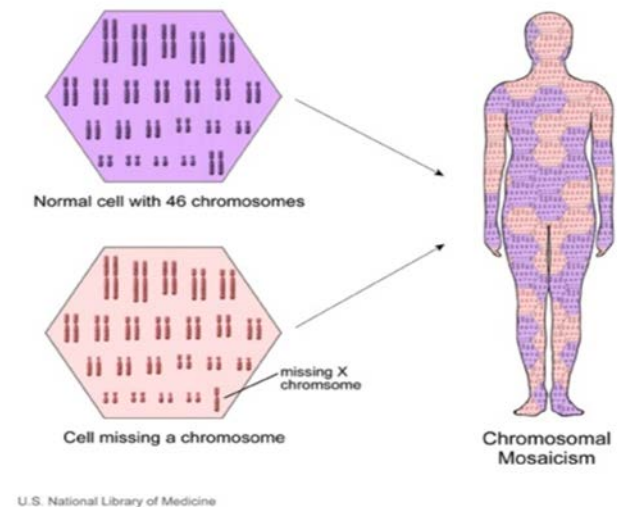
Feto-placental mosaicism

- Circulating cfDNA is from the placenta. Mosaicism affecting the placenta can lead to misleading cfDNA results as the placenta and fetus differ.
- Confined placental mosaicism (CPM) can lead to a false positive cfDNA result and diagnostic CVS test result.
- Consideration could be given to an amniocentesis test when positive cfDNA results are seen in a structurally normal fetus with low-risk combined or quadruple testing results.
- Isolated fetal mosaicism would always give false negative cfDNA results.



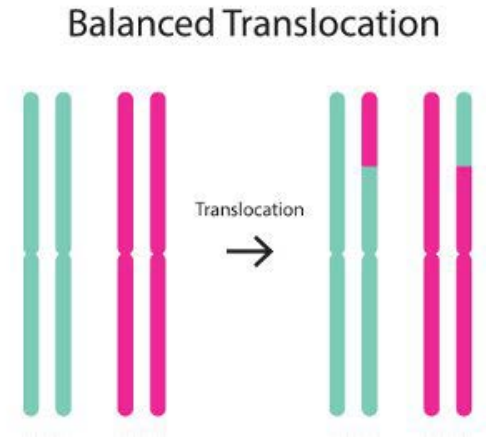
Maternal duplication or mosaicism

- If the patient reports a duplication or mosaicism, or is affected with the full trisomy, it is likely to produce a false positive result due to the excess chromosome material in the plasma.
- If there is a maternal translocation/rearrangement of a chromosome, then there is no contraindication to have the cfDNA test. This is because there is no extra DNA material present to affect the cfDNA assay.



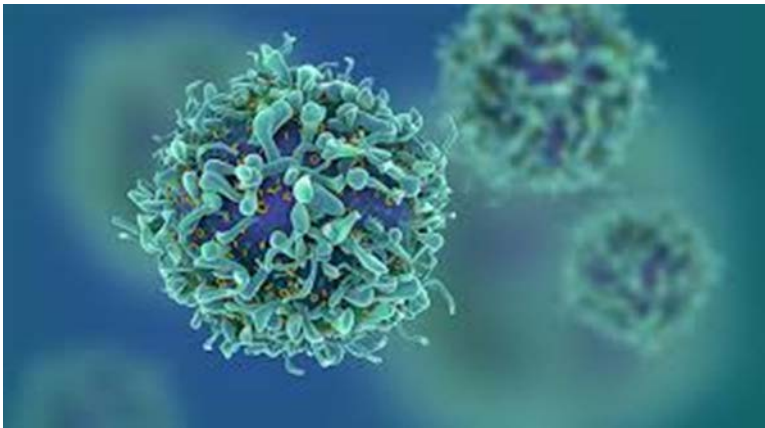
Balanced Translocation

- Balanced translocation is where a section from one chromosome swaps places with a section from a chromosome of another pair.
- 1 in 560 people have a balanced translocation. Those looking to have children need to be aware of the chance of an 'unbalanced' translocation, which may cause fertility problems, miscarriage, physical abnormalities or learning disabilities.
- NIPT is unable to report unbalanced transfers and thus CVS/amniocentesis is recommended.
- Patients reporting a balanced translocation should be referred for further genetic and/or fetal medicine consultations.



How can cancer affect a result?

- A tumour can shed tumour cfDNA into the maternal circulation.
- Cancer cells have major chromosome abnormalities that can be identified by cfDNA screening as a false positive high-chance fetal result.
- Pregnant mothers with cancer are advised against having an NIPT screening test.



False negative results

- A false negative result occurs when a pregnancy is thought to be low-chance for a trisomy following a screening test, but later in pregnancy it is discovered that the baby is affected with a trisomy.
- Amniocentesis is the only testing method that can provide a definitive diagnostic result. A patient must be aware of this.
- False negative results can occur due to:
 - use of incorrect a-priori risk
 - twin pregnancy
 - discordant mosaicism between the placenta and fetus (the fetus is affected but the placenta is not)



Fetal Anomalies and NIPT

- An ultrasound is recommended before any NIPT screening.
- If a high NT (nuchal translucency) is measured ≥ 3.5 mm, an NIPT is not recommended.
- The expectant parents should be made aware that the finding of a fetal structural anomaly is an indication for invasive testing with PCR and microarray CGH analysis.
- Fetal structural defects are associated with a higher chance of a non-aneuploid fetal chromosomal anomaly.
- In one study, 225 fetuses had an $NT \geq 3.5$ mm. In 24 of these pregnancies, a chromosomal anomaly other than a trisomy was detected.

It is important that women have a clear understanding of the limitations of cfDNA testing with fetal structural anomalies.

Sex chromosomal aneuploidy (SCA's)

- Some commercial NIPT's offer sex aneuploidy screening. Studies have reported a lot of variation in the effects of SCA conditions. People affected are still undiagnosed in the general population.
- Sex determination can lead to incidental findings of sex aneuploidy that may have minimal significance and cause no harm or disability to a fetus.
- Until sex aneuploidy screening becomes part of routine clinical practice, this additional testing by cfDNA is not recommended.
- False positive rates for sex aneuploidy screening remain high – thereby increasing the need for additional invasive testing.
- The recommendations by most leading institutional bodies (ACOG, RCOG, Nuffield Council on Bioethics and NSC) advise against screening for sex aneuploidy unless there is a clinical indication.

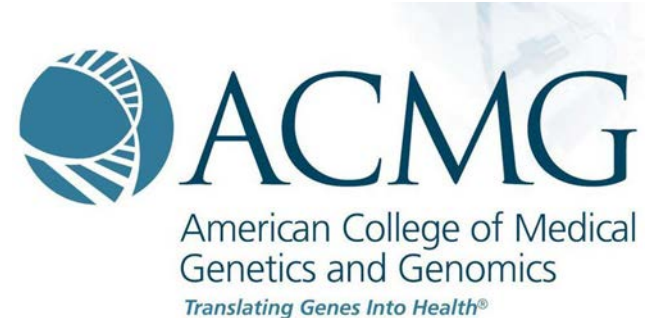


Screening for Microdeletions or Microduplications

- Advances in technology have created opportunities to improve and expand genetic testing.
- There has been a significant commercial interest in implementing these tests into routine clinical care.
- This creates challenges for staff and patients, including misuse, unintended consequences and misunderstanding.
- The SAFE test only screens for Down's, Edwards' and Patau's syndrome.

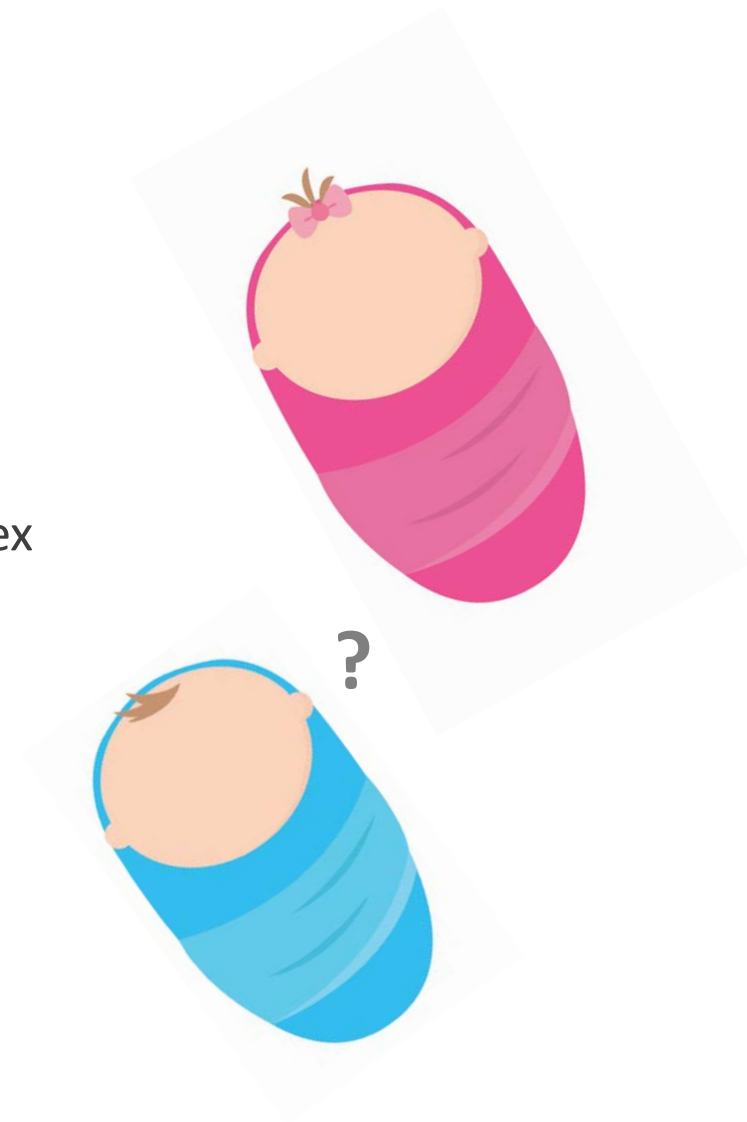
Screening for microdeletions or microduplications

- The SMFM and ACMG both highlight the fact that routine cfDNA screening should not be performed for microdeletions and microduplications.
- Most of the 'normal' population carry multiple small copy number variations. With this in mind, testing for other variations can lead to a high false positive rate with an associated increase in invasive testing.
- The latter goes against the principal of screening in pregnancy, which is to reduce invasive testing rates and parental anxiety.



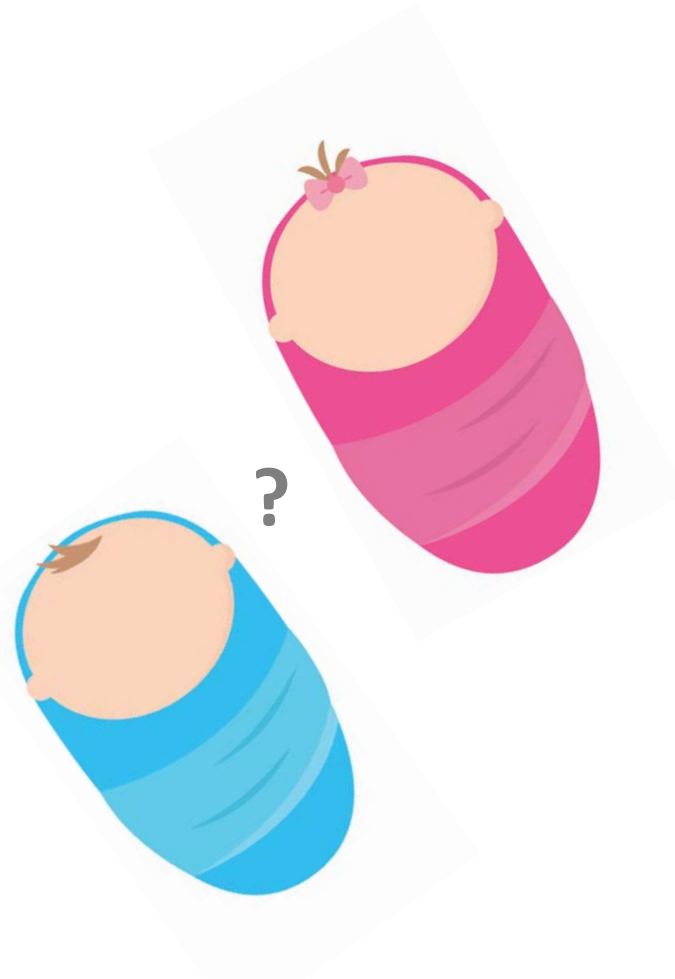
Fetal sex determination

- Most couples wishing to find out the sex of the fetus can be informed at the anomaly scan performed between 18-22 weeks.
- The majority of the motivation to find out the sex of the baby is for preparation and curiosity.
- Sex determination with NIPT is not possible for dichorionic twins.
- However, it is sometimes important for certain genetic conditions, to know the sex of the fetus (sex-linked conditions).



Ethical considerations on fetal sex determination and NIPT

- In March 2017, the Nuffield Council on Bioethics published a report that advises the elimination of sex determination in the private sector.
- There are concerns that using cfDNA tests in early pregnancy can lead to sex-selective termination.
- In 2015 The Department of Health stated that termination on the grounds of fetal sex is illegal.
- This is not applicable for patients with inherited genetic conditions.
- These patients will have genetic counselling and support to discuss the options available to them.



When is sex determination recommended?

- Sex determination is often requested by genetic teams prior to diagnostic testing for sex-linked conditions.
- A sex-linked condition is described as an alteration in one gene on the X chromosome, and causes a condition in males. Examples include Haemophilia and Duchenne muscular dystrophy.
- cfDNA testing can be used to determine the sex of the fetus when medically indicated. This gives patients the opportunity to decide if an invasive test is beneficial.

