Module 7:
Delving deeper

St George's University Hospitals NHS Foundation Trust

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

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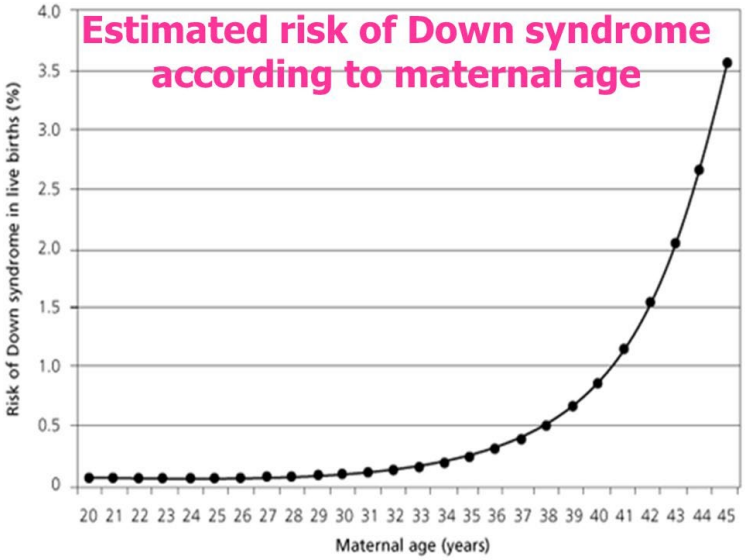
A *priori* risk (background risk)

This is the starting or background risk for a person having a screening test. Some cell-free DNA tests use the maternal age related risk, 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).
- 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.

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

- The SAFE test has the option to include the use of prior screening tests for risk calculation.
- Prior screening tests include the Combined test and the Quadruple test.
- This gives the most accurate, personalised and comprehensive prenatal screening result for pregnant women.
- If results for a prior screening test is unavailable, the default prior is the maternal age.

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SAFE screening report

TEST RESULTS:

TRISOMY	*BACKGROUND RISK (before the SAFE test)	SAFE TEST RISK SCORE	CLINICAL SUMMARY
Trisomy 21	1 in 38^{PT}	Greater than 95%	**HIGH CHANCE — INVASIVE TEST RECOMMENDED**
Trisomy 18	1 in 537^{PT}	Less than 1 in 1,000,000 (<0.0001%)	LOW CHANCE
Trisomy 13	1 in 537^{PT}	Less than 1 in 1,000,000 (<0.0001%)	LOW CHANCE

the SAFE test is indicated for screening NOT diagnosis — (results should be reviewed and discussed with the healthcare provider)

PT: Prior Test

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

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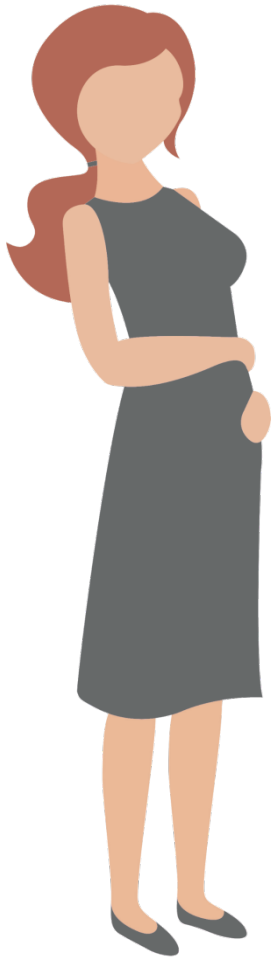
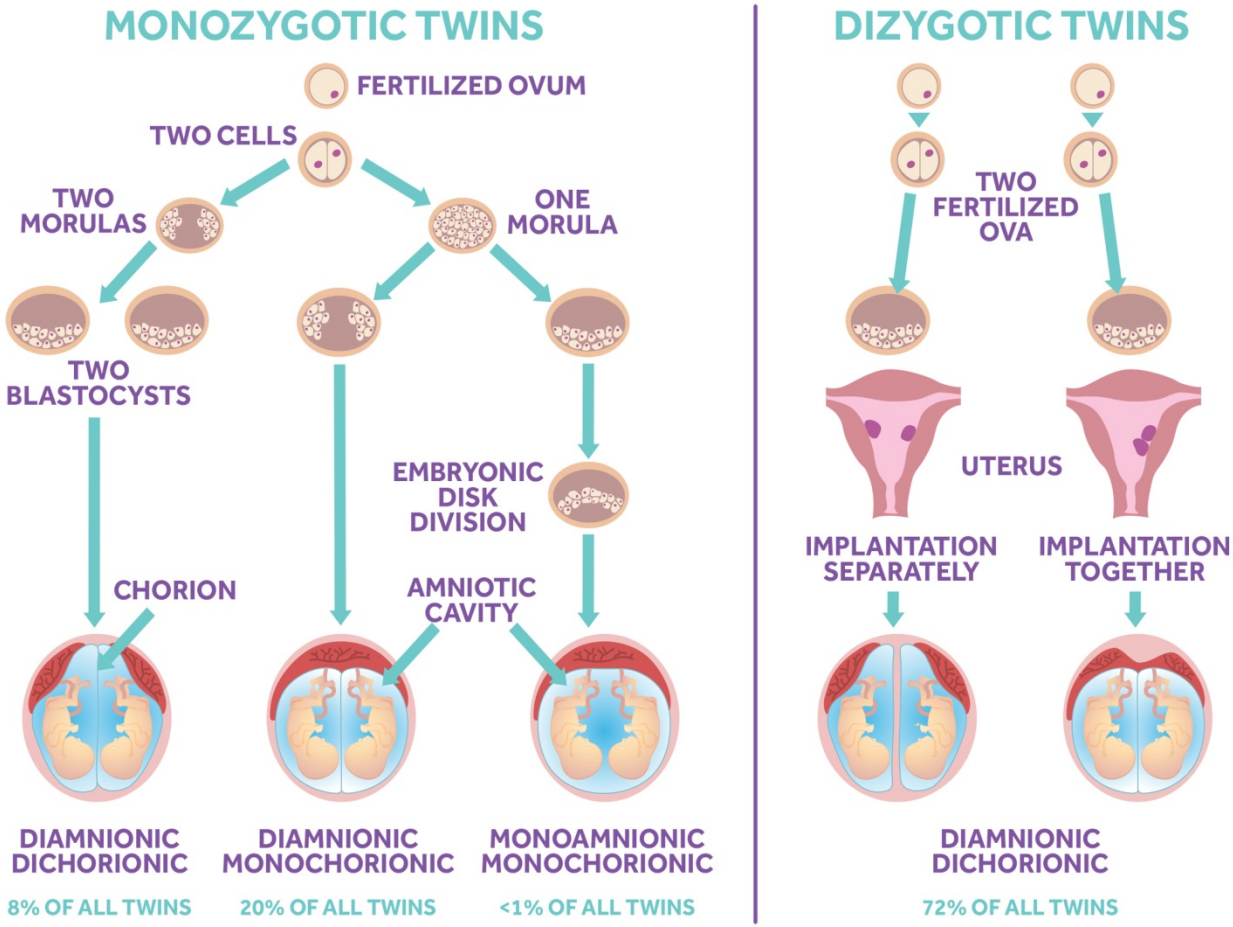
A priori risk case study

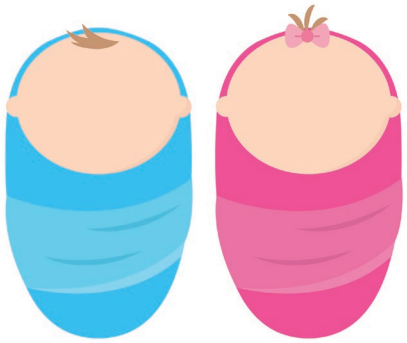
- A patient that is 21 years old would have a maternal age-related chance of 1 in 1450.
- When the sample is tested, the cfDNA result may halve the chance, giving a result of 1 in 2900 (low chance).
- If the same patient had the first trimester combined test (FTCT) first, and the result was 1 in 5; the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



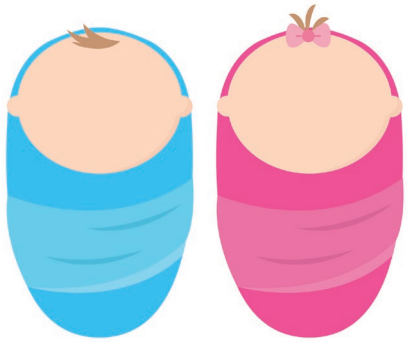
NIPT in twins



- 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.

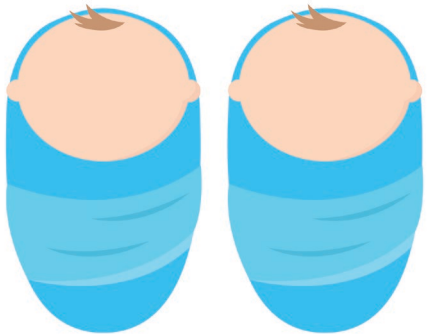
NIPT in dizygotic twin pregnancies

- 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 twins 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

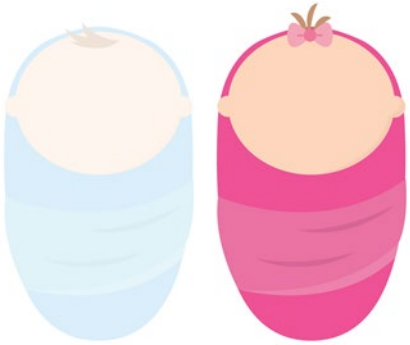
- The SAFE test is suitable for twin pregnancies. However, a high chance trisomy NIPT result in twins cannot determine which twin is affected.
- Performance of cfDNA screening in monozygotic (identical) twins is the same as for singleton pregnancies.



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.

- Vanishing twins occur in up to one out of every eight multi-fetus pregnancies and may not even become known in most cases.



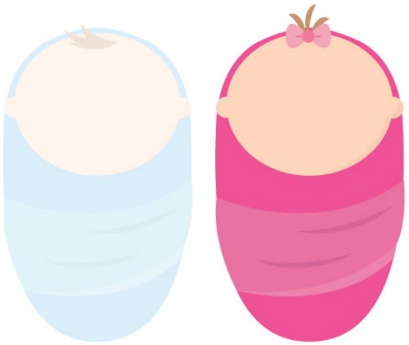
Vanishing twins and NIPT

- 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:

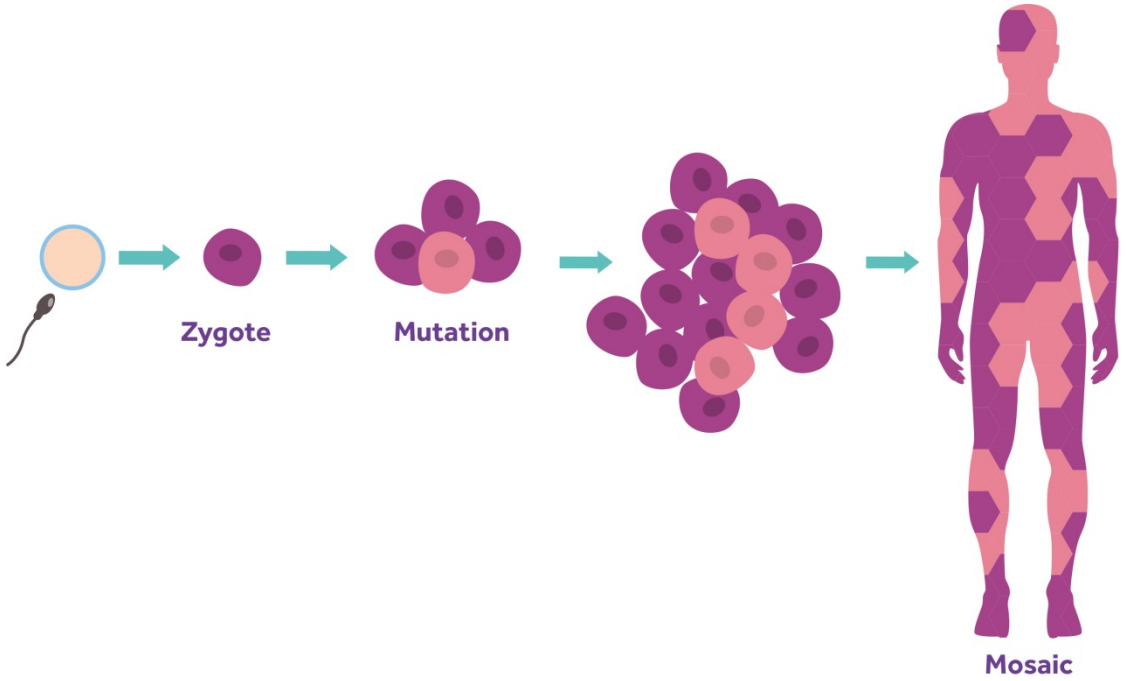
- Vanishing twin
- Placental mosaicism

Conditions affecting the mother:

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

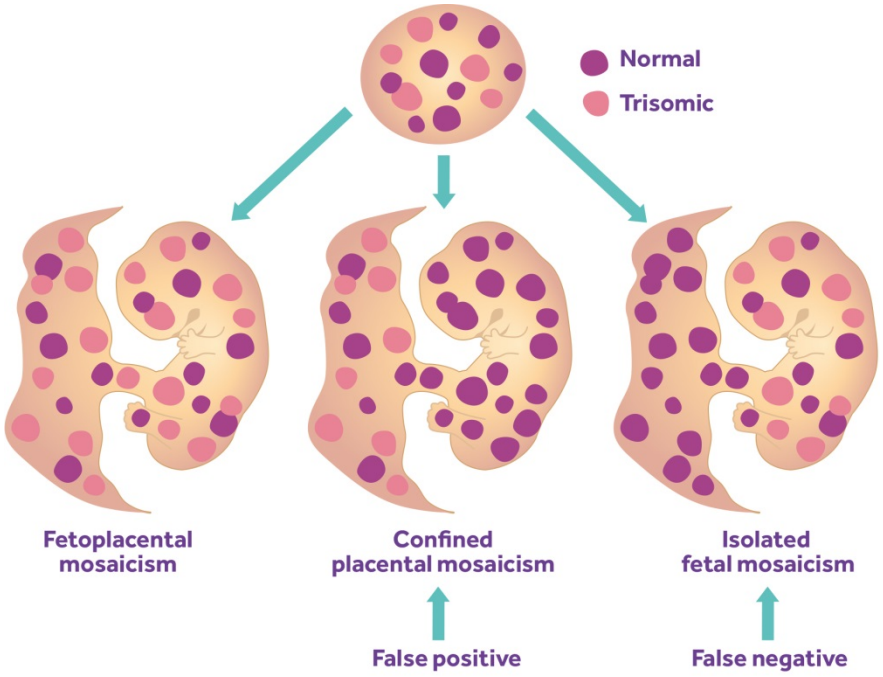
Mosaicism

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 chance combined or quadruple testing results.
- Isolated fetal mosaicism would always give false negative cfDNA results.



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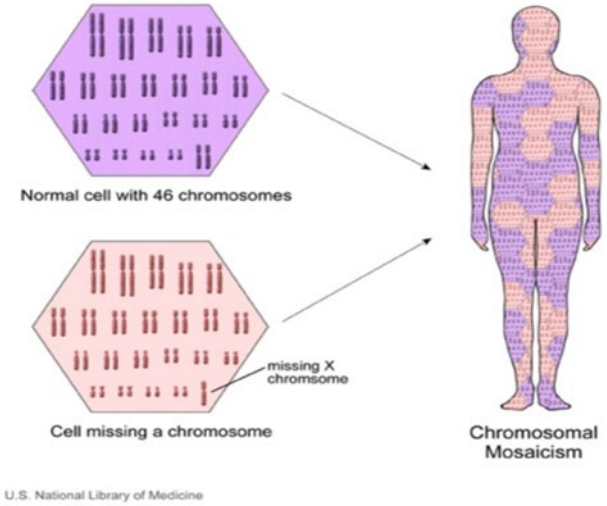
Maternal duplication or mosaicism

- 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.



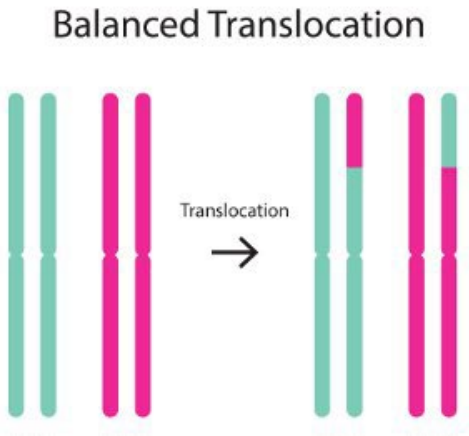
Balanced Translocation

- 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.

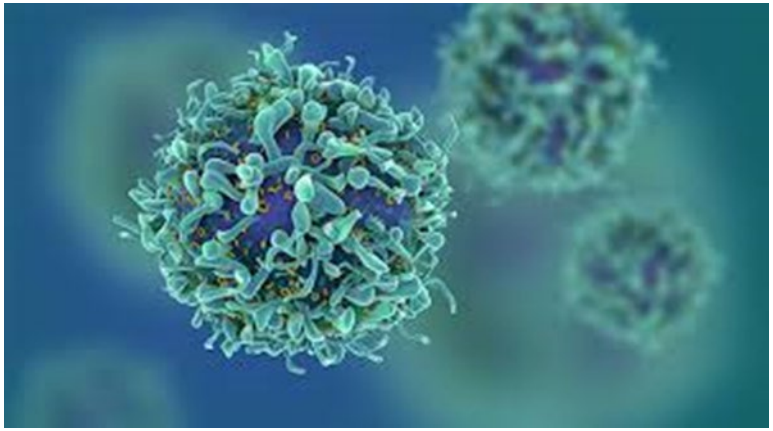
- NIPT is unable to report unbalanced translocations and thus CVS/amniocentesis is recommended.

- 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 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 abnormality.
- In one study, 225 fetuses had an NT≥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.

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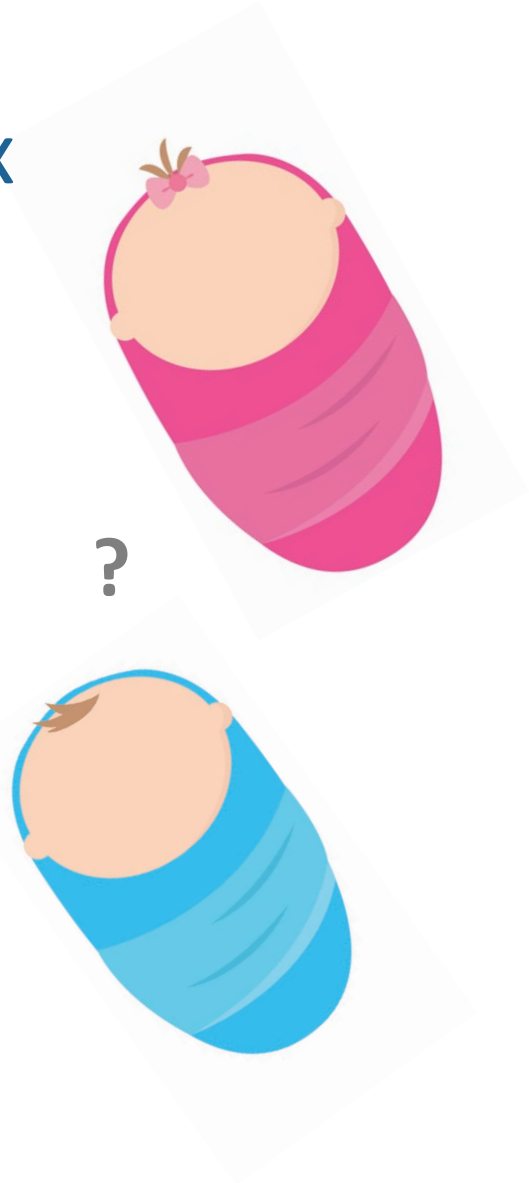
Fetal sex determination

* not offered through NHS Trusts

- 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.
- 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.

- It was advised that NIPT should not normally be used to test whether a fetus has a less significant medical condition or impairment; or to find out whether the fetus is a carrier of a gene, nor reveal non-medical traits, including sex.

- Sex determination is not offered by the SAFE test within NHS trusts in line with these recommendations.

- This is not applicable for patients with inherited genetic conditions.

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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.
- These patients will have genetic counselling and support to discuss the options available to them.