Free fetal DNA in maternal plasma: application in multiple pregnancies

Laura Solé Font, **Universitat Autònoma de Barcelona**

Universitat Autònoma de Barcelona

Introduction

The technique based on free fetal DNA in maternal plasma is used as a non-invasive optional prenatal test that allows doing genetic studies from a single maternal blood sample. It has been implemented in hospitals to analyse pregnancies with a high risk of chromosomal trisomies, as well as to determine the fetal sex and rhesus blood group. The sensibility and false-positive rate of this method are 99% and 0,1% respectively.

Multiple pregnancies are associated with an increased spontaneous fetal loss and a higher rate of chromosomal abnormalities. Moreover, the number of multiple pregnancies has risen in recent years, probably related to the higher utilization of *in vitro* fecundation techniques. A higher fraction of these pregnancies are from women with an advanced age, who are also at an increased risk of chromosomal abnormalities. For these reasons, these women are reluctant to be subjected to an invasive prenatal test.

To sum up, the application of this technique in multiple pregnancies would be very useful in order to avoid doing an invasive test, which also increments the probability of an abortion. Many research groups are investigating what method based on the already implemented process would be more appropriate when there is more than one fetus.

Materials and methods

I've done a bibliographic research using articles and reviews contained in the PubMed database. I have preferably chosen recent articles about this topic, most of them from 2011 and later, with a few exceptions.

Keywords: free fetal DNA in maternal plasma, multiple pregnancies, prenatal diagnostic, aneuploidies, MPS.

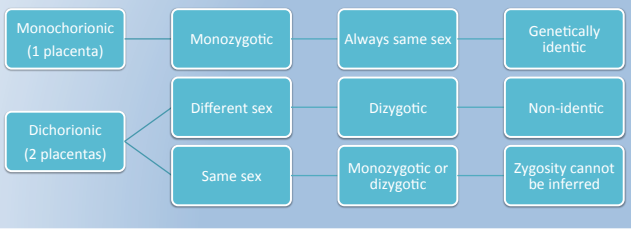
This work aims to analyse the possibility of implementing the free fetal DNA-based technique as a secondary prenatal test in multiple pregnancies and its limitations. For this purpose, I propose a possible approach of this technique.

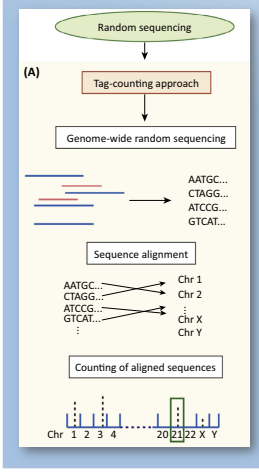
1. Fetus classification

- **Chorionicity:** depends on placentation. It can be only determined non-invasively on the first trimester by ultrasounds.
- **Zygosity:** refers to the genetic identity of each twin. It has to be inferred from chorionicity.

Fetal DNA derives from placental trophoblastic cells. As there could be mosaicism between placenta and fetus, the chorionicity determination could allow better diagnosis, although it's not currently used.

Furthermore, it has not been shown any relationship between chorionicity and fetal DNA concentration.





2. Maternal plasma analysis

1. **Total DNA quantification:** absorbance at 260nm or ß-globin quantification by RT-PCR of maternal plasma. It's used to ensure the quality of the collected DNA.
2. **Massive parallel sequencing (MPS):** to analyse fetal DNA.

MPS is the most used sequencing method. Other techniques that could be used are:

- **Digital PCR:** several simultaneously amplifications. High fractional fetal DNA is needed. It's unlikely to be used on multiple pregnancies.
- **Targeted sequencing:** SNP- or non-SNP-based approaches. They provide fast and accurate results and could be an adequate alternative method.

Figure 1. MPS procedure. Extracted from: Wong A & Lo D (2015).

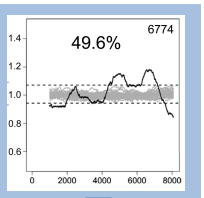
Dizygotic twins:

SNP 1 Mother: AA Fetus I: AB Fetus II: AB

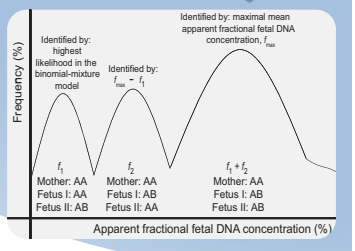
SNP 2 Mother: AA Fetus I: AA Fetus II: AB

$$f = \frac{2p}{p+q}$$

$$f1 \neq f2$$



- **Monozygotic twins:** apparent fetal DNA concentrations would be equal in all the informative SNPs (1). Their mean (for every 1000 informative SNPs on contiguous genomic blocks) would demonstrate no regional variation (2).



Figures 2 and 3. Zygosity determination. Extracted from: Qu, J. Z. Z. *et al* (2013).

3. FetalQuant algorithm

SNP loci homozygous in the mother but heterozygous in at least one fetus.

1. Apparent fetal DNA concentration (f).
2. Zygosity determination by regional variation.
3. **In dizygotic twins:** fetal DNA concentration for each twin fetus.

$$\%chrT = \frac{Unique\ count\ for\ chrT}{Total\ unique\ count}\ x\ 100\%$$

$$Correction\ for\ GC\ content$$

$$chrT\ z\_score = \frac{(\%chrT - \%chrT_{ref})}{S.D.\%chrT_{ref}}$$

$$z\_score > 3 = trisomy\ of\ chrT\ in\ at\ least\ one\ fetus$$

$$\%chrT = \%chrT_{ref} + 0{,}5f \cdot \%chrT_{ref}$$

Additional step in dyzigotic fetus

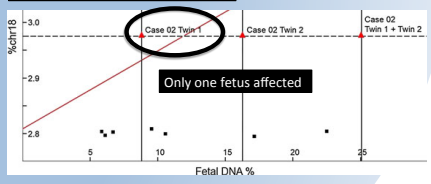


Figure 4. Determination of the number of fetuses with trisomy. Extracted from: Leung, T. Y. *et al* (2013).

4. Detection of trisomies

1. **Genomic representation of each chromosome (chrT).**
2. **Correction for GC content.**
3. **ChrT z-score:** >3 indicates trisomy in at least one fetus.
4. **In dizygotic fetus:** to determine the number of affected fetuses.

Conclusions

1. The present approach allows quantifying total and fetal DNA in maternal plasma as well as the fetal fraction corresponding to each fetus. In addition, it enables the correct classification of the fetus. Consequently, it's possible to choose the better aneuploidy test, depending on zygosity.
2. The trisomy detection rate for 21, 13 and 18 chromosomes is around 94% with a false-positive rate of 0%. However, it's necessary to do studies with a high number of cases following the complete process, even though the results are promising.
3. The cost-effectiveness analyses of this technique applied in single pregnancies, which are at high risk of trisomies, have demonstrated that it's appropriate as a secondary optional prenatal test. In multiple pregnancies these analyses still remain.
4. In order to implement this technique in multiple pregnancies, it's essential to study its advantages performing the complete process in real clinical cases, where all samples have to be collected and analysed immediately without storage.

References

1. Wong, A. I. C. & Lo, Y. M. D. Noninvasive fetal genomic, methylomic, and transcriptomic analyses using maternal plasma and clinical implications. *Trends Mol. Med.* **21 (2),** 98–108 (2015).
2. Leung, T. Y. *et al.* Noninvasive twin zygosity assessment and aneuploidy detection by maternal plasma DNA sequencing. *Prenat. Diagn.* **33,** 675–681 (2013).
3. Qu, J. Z. Z. *et al.* Noninvasive prenatal determination of twin zygosity by maternal plasma DNA analysis. *Clin. Chem.* **59 (2),** 427–435 (2013).