Genetic Analysis of Early and Recurrent Pregnancy Loss: Challenges and Advances

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Introduction

Pregnancy loss, defined as the spontaneous termination of pregnancy before fetal viability, affects around one in four pregnancies and often carries significant emotional and clinical consequences. Genetic abnormalities, particularly chromosomal aneuploidies such as autosomal trisomy, monosomy X, and polyploidy, are implicated in up to 70% of first-trimester miscarriages, typically arising from *de novo* meiotic errors.[1,2]

Genetic analysis of products of conception (POCs) is vital for understanding the causes of miscarriage and informing future reproductive care. While conventional karyotyping remains the standard approach, it is limited by culture failure, maternal cell contamination (MCC), and low resolution. Molecular methods such as chromosomal microarray (CMA), QF-PCR, and SNP arrays provide higher accuracy but still face technical challenges, especially when tissue quality is poor.[3,4] Distinguishing between euploid and aneuploid losses is clinically important, as euploid miscarriages are more likely linked to underlying maternal health conditions and carry a greater risk of recurrence. However, ploidy assessment is often hindered by difficulties in obtaining suitable tissue. The presence of cell-free fetal DNA (cffDNA) in maternal blood offers a promising, non-invasive alternative. Though widely used in prenatal screening, its application in miscarriage remains under investigation. Preliminary studies show encouraging results but require larger-scale validation.[5,6,7]

This study presents our recent laboratory experience in investigating pregnancy loss using various sample types and genetic testing methodologies, with a particular focus on the feasibility and diagnostic potential of cell-free DNA (cfDNA) testing.

Methods

QF-PCR and SNP-array

DNA from POCs was extracted using a homemade salting-out procedure. Detection of common aneuploidies was performed using the Devyser Complete kit (Devyser AB, Stockholm, Sweden). All samples with a normal female result were tested for maternal cell contamination (MCC) by comparing multiple microsatellite markers between maternal blood and DNA obtained from the POC sample. Only samples confirmed to be free of MCC were subsequently analysed by CytoScan 750K array (Thermo Fisher Scientific, Santa Clara, CA). Data analysis was performed using Genotyping Console™ v4.0 and Chromosome Analysis Suite (ChAS) v4.5.0.34, with annotation based on NetAffx™ 20250201 (UCSC hg19 reference genome).

Genome-wide analysis in cfDNA

Maternal blood was collected in 10 mL Cell-Free DNA BCT CE tubes (STRECK, La Vista, NE) while the pregnancy was ongoing. Fetal aneuploidy screening was performed using cfDNA extracted and analysed with the commercial platform VeriSeq NIPT™ Solution v2 (Illumina, Foster City, CA, USA). This technology employs whole-genome paired-end sequencing to assess aneuploidy status across all chromosomes, and it also enables the detection of partial duplications and deletions involving any autosome.

Results

POCs analysis

Since 2022, a total of 22 POC samples have been received in the laboratory. In eight cases (36.4%), it was not possible to obtain a conclusive result due to MCC. An abnormal result was identified in nine cases (40.9%). QF-PCR revealed six chromosomal abnormalities: four triploid cases and two cases of chromosome 13 trisomy. Additionally, SNP-array analysis identified three further rare trisomies, as illustrated in Figure 1A.

cfDNA analysis

In 2025, we began accepting blood samples from women with retained miscarriage for genome-wide cfDNA analysis. To date, 15 samples have been analysed. In two cases (13.3%), no result could be obtained due to failure in the quality control of the sequencing data. Aneuploidy was detected in 10 cases (66.7%), as shown in Figure 1B.

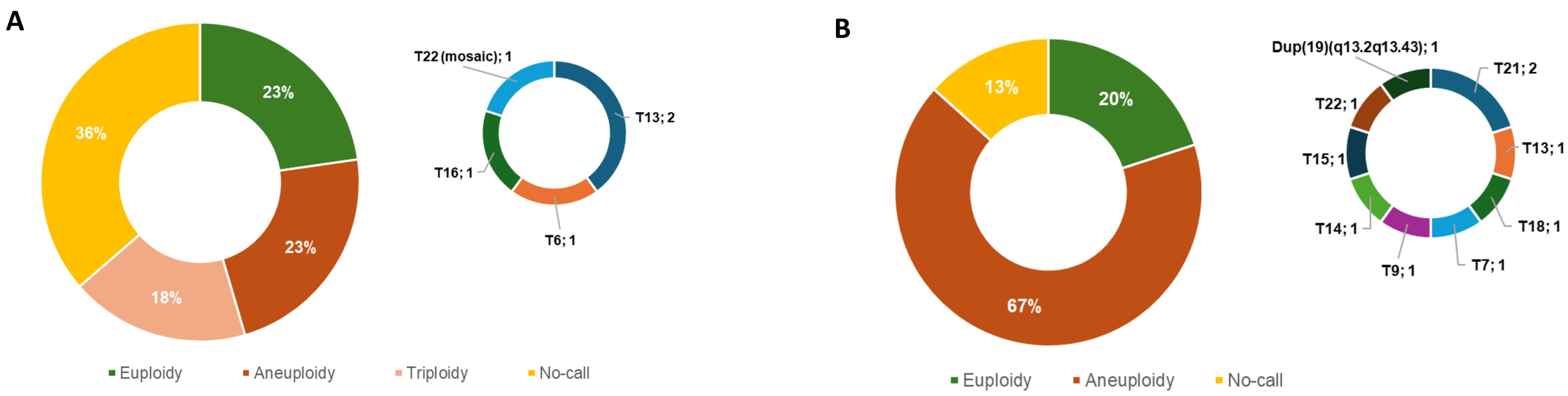

Figure 1: Distribution of tests results. **A)** Results obtained in the POC samples (by QF-PCR and SNP-array) and **B)** Results obtained in genomewide cfDNA analysis.

Only in two cases were possible to obtain both maternal blood sample (collected in a Streck tube prior to expulsion) and the corresponding POC sample. Unfortunately, in one of these cases, no result could be obtained from either sample, due to poor sequencing data quality in the cfDNA and maternal cell contamination in the POC. In the other case, cfDNA analysis detected trisomy 21, while POC analysis revealed triploidy with an extra chromosome 21. This discrepancy can be explained by a methodological limitation of our genome-wide cfDNA analysis platform, which is unable to detect polyploidy.

Discussion and Conclusion

The study of fetal loss causes is a valuable resource for those facing this situation, which, although common, is emotionally challenging and can even be traumatic. Understanding the underlying cause can provide psychological relief to the woman by reducing feelings of guilt, as well as helping to estimate the risk of recurrence and supporting informed reproductive choices. However, the collection of tissue from the product of conception is a technically difficult procedure, often compromised by significant contamination with maternal material, which frequently prevents a conclusive analysis.

Ideally, the recommended methodology would be to obtain a karyotype of the conceptus. However, in most cases, this is not feasible due to the challenges associated with establishing the necessary cell culture. As an alternative, the use of QF-PCR and SNP-array techniques to analyze the conceptus DNA offers a practical solution. These methods do not require tissue culture and provide higher resolution; however, they are unable to detect balanced chromosomal rearrangements, which, although rare, may be the cause of fetal loss in a small proportion of cases, particularly in recurrent miscarriage.

In recent years, several studies have highlighted the potential role of cfDNA analysis as a complementary approach.[8,9] While it remains a screening tool, cfDNA has demonstrated improved performance in detecting aneuploidies and even in identifying parental chromosomal translocations.[10] Our experience supports these findings: the proportion of samples with MCC, that rendered the analysis impossible, was significantly higher than the rate of inconclusive results (“no-calls”) obtained through cfDNA analysis. However, it is important to note that our cfDNA methodology does not detect triploids or other polyploidies, which are relatively common in this clinical context.

Given that all methodologies have inherent limitations, employing two complementary approaches is the most effective strategy to maximize diagnostic yield in the management of early and recurrent miscarriages. The ideal protocol for investigating fetal loss would involve collecting a maternal blood sample for cfDNA analysis prior to the removal of embryonic tissue or the administration of medication. Following this, embryonic tissue should be collected under optimal conditions to allow for successful cell culture and DNA extraction. This approach enables rapid aneuploidy diagnosis, screening for MCC, SNP-array analysis, or karyotyping, depending on the results of the QF-PCR and the success of the tissue culture.

References

1. Quenby S. *et al.* Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet. 2021;397:1658-1667.
2. Blue NR, Page JM, Silver RM. Genetic abnormalities and pregnancy loss. Semin Perinatol. 2019 Mar;43(2):66-73.
3. Mcqueen DB, Lathi RB. Miscarriage Chromosome Testing: Indications, Benefits and Methodologies. Semin Perinatol (2018) 43(2):101–4.
4. Finley J. *et al.* The genomic basis of sporadic and recurrent pregnancy loss: a comprehensive in-depth analysis of 24,900 miscarriages. Reprod Biomed Online. 2022; 45(1):125-134.
5. Schlaikjær Hartwig *et al.* Cell-free fetal DNA for genetic evaluation in Copenhagen Pregnancy Loss Study (COPL): a prospective cohort study. The Lancet, 2023; 401:10378, 762 – 771.
6. D'Ippolito S. *et al.* Investigating the "Fetal Side" in Recurrent Pregnancy Loss: Reliability of Cell-Free DNA Testing in Detecting Chromosomal Abnormalities of Miscarriage Tissue. J Clin Med. 2023; 7;12(12):3898.
7. Kutteh WH. et al. Cell-Free DNA Analysis of Fetal Aneuploidies in Early Pregnancy Loss. J Clin Med. 2024; 23;13(15):4283.
8. Pauta M. *et al.* Genome-Wide Cell-Free DNA Analysis for Aneuploidy Detection in Miscarriages: Test Performance Meta-Analysis. Prenat Diagn. 2025; 27.
9. Della Valle L. et al. Accuracy of cell-free fetal DNA in detecting chromosomal anomalies in women experiencing miscarriage: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2025;65(1):13-19.
10. van Zutven LJCM *et al.* What proportion of couples with a history of recurrent pregnancy loss and with a balanced rearrangement in one parent can potentially be identified through cell-free DNA genotyping? Mol Cytogenet. 2023; 29;16(1):26.