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Fertility centers and cell-free DNA screening (cfDNA): a review of one lab's cfDNA screening performance in the reproductive health setting

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1. Introduction

According to the Center for Disease Control and Prevention, there were 73,831 live births conceived using assisted reproductive technology in 2018.[1] Additionally, more individuals conceive pregnancies through other types of fertility treatments. Research has shown that traditional serum screening for aneuploidy in pregnancy is less effective in IVF conceptions, with increased false positive rates.[2] Patients who conceived through IVF/ICSI are also less likely to opt for prenatal diagnosis, even after controlling for confounding variables.[3] Given the risk of procedure related pregnancy loss from amniocentesis and CVS, patients may request cell-free DNA screening (cfDNA) to provide additional information prior to deciding on prenatal diagnostic testing. Massively parallel sequencing (MPS) allows for screening of maternal plasma specimens for aneuploidy regardless of means of conception or biologic source of oocyte genetic contribution. We describe the clinical laboratory experience of one lab running 4,018 consecutive cfDNA samples sent by offices reported to specialize in Reproductive Endocrinology and Infertility (REI).

2. Methods

Over 4,000 maternal blood samples submitted for cfDNA screening from providers identified as specializing in REI are included in this cohort. Samples were subjected to DNA extraction, library preparation, and genome-wide MPS as described by Jensen et al.[4] Outcome data was obtained from two sources. First, ad hoc provider feedback, when available, was reviewed. Second, data was cross referenced with an internal database of microarray/ karyotype diagnostic results from CVS, amniocentesis and POC samples. Statistical analysis of this cohort was completed and compared to a larger cohort of >200,000 cfDNA samples run on assay version 5 (AV5), the same assay version as the REI cohort. Study data was statistically described using counts, rates and measures of central tendency. A two by two contingency table was used to calculate sensitivity, specificity, and positive predictive value (PPV). VassarStats was used to complete two sample t tests to compare laboratory and demographic metrics between the REI and >200,000 cfDNA AV5 cohorts, assessing the average turnaround time, the non-reportable rates, average maternal age, the average gestational age, and the average fetal fraction. A *p* value <0.05 was considered statistically significant.

3. Results

Of the 4,018 samples, 97.6% were reported as singletons and 2.4% multifetal. 43 results were positive for trisomy 13, 18 or 21, with a positivity rate of 1.07%. The positivity rate for overall AV5 cohort was 1.12%. The most common result was trisomy 21 (n=29) followed by trisomy 18 (n=7) and trisomy 13 (n=7). Of the 43 positive results there was 1 reported false positive for trisomy 13 and no reported false negatives (**Table 1**). Mosaicism ratio (MR) was assessed for all positive results. As previously described by Rafalko et al, mosaic sequencing data and a depressed MR can be associated with reduced positive predictive values due its association with certain biological events which may lead to discrepancies between cfDNA results and diagnostic testing.[5] This confirmed false positive Trisomy 13 case had low mosaic chromosome 13 sequencing data and the history of a co-twin demise with a healthy live singleton birth later confirmed.

A two sample t test was performed, there was a significant difference in maternal age of the REI cohort (M=33.7 years, SD=5.44) and the larger assay version 5 cohort (M=32.1, SD=5.98); t(7404)=18.43, p<.00001. Of note, the average fetal fraction was not significantly lower (M=0.85, SD=.034) than the larger cohort (M=0.87, SD=.035); t(0)=-3.8, p=0.5, even though this population has a greater likelihood of health conditions requiring medications that may impact fetal fraction (i.e. Lovenox). The turnaround time averaged 2.8 calendar days. (**Table 2**)

Of the positive results (**Table 3**), maternal age is clearly higher in the positive group than the euploid group. Previous studies by other laboratories[6] have shown trisomy 13 and trisomy 18 pregnancies have lower fetal fraction than euploid and trisomy 21 pregnancies, which may impact the ability to return results. This laboratory's previous study[7] on non-reportable results due to low fetal fraction did not see an enrichment for aneuploidy, specifically trisomy 13 and 18, in non-reportable samples. For this REI cohort while fetal fraction is somewhat lower in the T18 and T13 samples versus T21 and euploid samples, it is not approaching the minimal bounds of fetal fraction required for reporting. (**Table 3**).

4. Conclusions

Patients who conceive a pregnancy through REI can experience financial and/or emotional burdens that may influence their views on diagnostic procedures. The performance of MPS cfDNA screening in the REI population is on par with the experience seen in the >200,000 AV5 cohort.[8] The reporting of mosaicism ratio can provide additional insights for IVF/REI pregnancies by presenting the most personalized interpretation of positive results. As long as the appropriate platform is chosen, cfDNA can be a useful screening tool in this population when risk mitigation is most desired.

Key Points:

- cfDNA screening in REI conceived pregnancies demonstrates strong performance for common aneuploidies
- Mosaicism ratio provides a more personalized interpretation of positive results for REI conceived pregnancies
- Given the appropriate testing platform, cfDNA is a useful screening tool for REI conceived pregnancies

Tables

Table 1: Performance based on ad hoc feedback and extensive cytogenetic cross reference review

Chromosome	Number of cases reported negative	Number of cases reported positive	Number of false negatives reported	Number of false positives reported
Trisomy 21	3,933	29	0	0
Trisomy 18	3,955	7	0	0
Trisomy 13	3,955	7	0	1

Aneuploidy	Relative Observed Sensitivity	Relative Observed Specificity	Relative Observed PPV
Trisomy 21	>99.9%	>99.9%	>99.9%
Trisomy 18	>99.9%	>99.9%	>99.9%
Trisomy 13	>99.9%	99.97%	85.7%

Table 2: cfDNA laboratory experience comparing REI referred samples to the 8-Years' Experience Cohort of >200,000 samples

Laboratory Performance Metrics	REI Cohort	Number of cases reported positive	Number of false positives reported
Average Turnaround Time (calendar days)	2.8	2.8	Not Statistically Significant p=0.5
Non-reportable: Technical and QNS	1.3%	1.06%	Not Statistically Significant p=0.582
Mean Maternal Age (years)	33.7	32.1	**Statistically Significant p=<0.00001**
Mean Gestational Age (weeks)	12.3	13.4	Not Statistically Significant p=0.5
Average Fetal Fraction	8.5%	8.72%	Not Statistically Significant p=0.5

Table 3: MPS cfDNA laboratory experience of screened negative and screened positive samples

Laboratory Performance Metrics	Euploid n=3919	Trisomy 21 n=29	Trisomy 13 n=7	Trisomy 18 n=7
Average Turnaround Time (calendar days)	2.7	3.4	3.6	4.0
Mean Maternal Age (years)	33.6	36.2	37.4	37.3
Mean Gestational Age (weeks)	12.3	13.6	11.0	13.4
Average Fetal Fraction	8.6%	8.1%	7.3%	6.8%

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26th ISPD Annual Conference; 2022 June 19-23

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