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cfDNA screening in triplets: a clinical laboratory experience

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I. Abstract

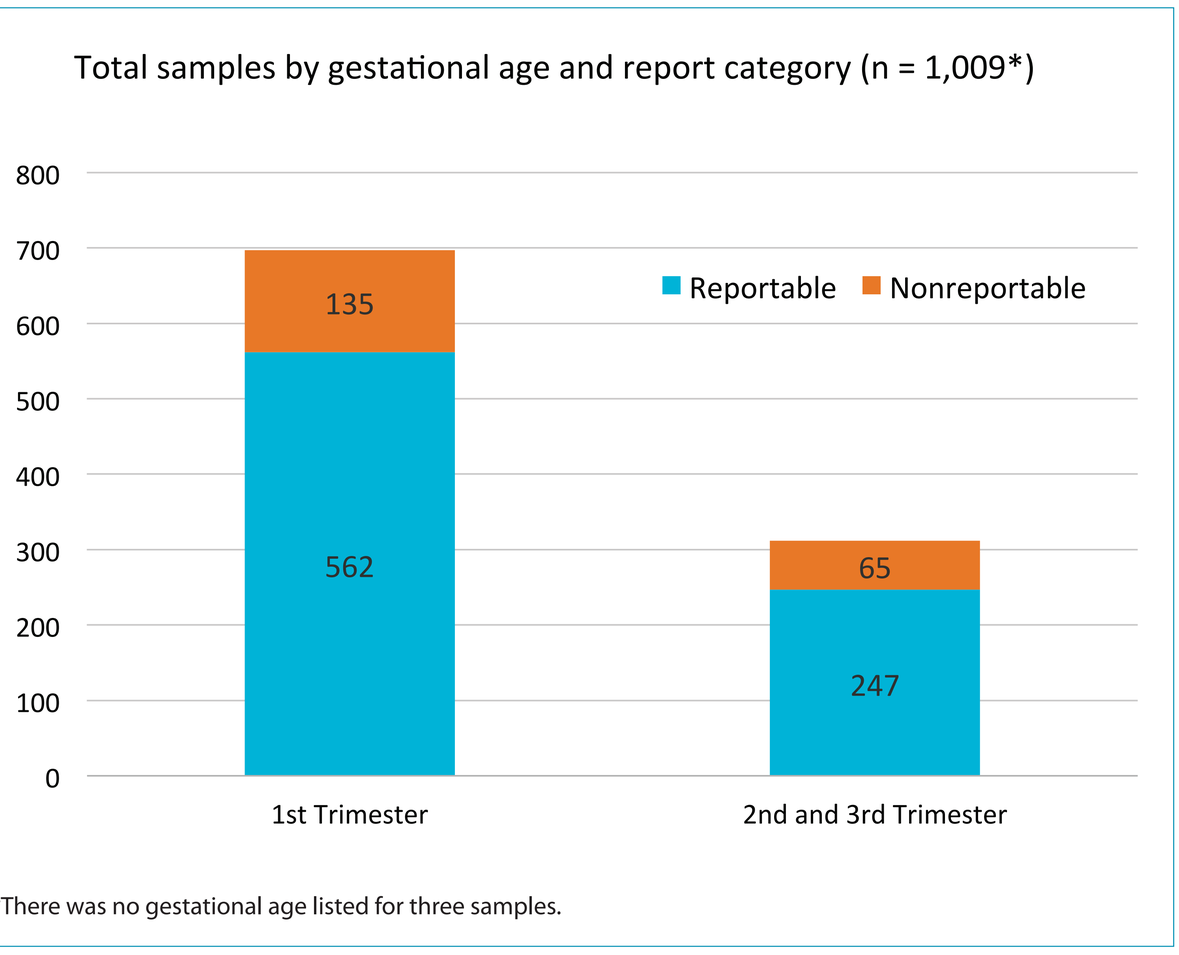
Cell-free DNA (cfDNA) aneuploidy screening in multifetal pregnancies has been validated and clinically available since 2012.[1] Between 1980 and 2009, the rate of triplets and high-order multiple births has increased more than 400%.[2] Over 1,000 samples from triplet gestations have been submitted for screening to one laboratory. The lack of conventional biochemical screening in triplets and increased risk for pregnancy complications with invasive testing positions cfDNA screening as a valuable alternative. The laboratory experience of the MaterniT® 21 PLUS test in triplets will be described.

II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® 21 PLUS testing were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as previously described by Jensen et al. Sequencing data were analyzed to detect autosomal trisomies and other subchromosomal events as described by Zhao et al. Fetal fraction requirements were adjusted in proportion to fetal number (e.g. three times minimum singleton fetal fraction threshold for triplets). Data analysis was performed on samples submitted for testing with a fetal number of 'three' indicated on the test requisition. Outcome data were collected by phone or email from the ordering provider.

III. Results

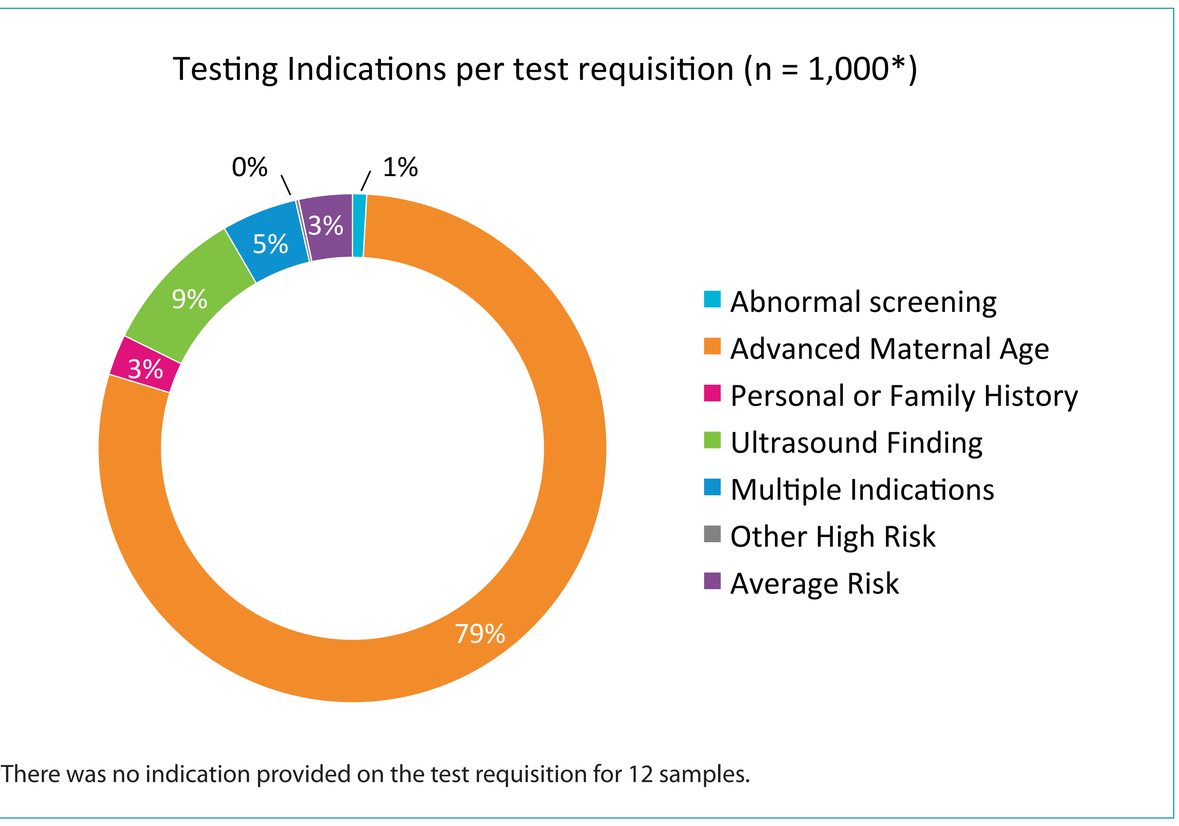
From 2013 through July 2019, 1,012 triplet samples were submitted for cfDNA screening. The average turnaround time was 5.5 calendar days. Average turnaround times have decreased with assay enhancements; in triplet samples resulted in 2018 and 2019 the average turnaround time improved to 3.5 calendar days. Most triplet samples were submitted in the first trimester (69%) and very few (n= 9, 1%) were submitted in the third trimester. The majority of samples (80%) were successfully reported as a positive or negative result. The average fetal fraction of reported samples was 13.5%. In triplet samples that were not reported due to insufficient fetal fraction, 19.5% of all triplet samples, the average fetal fraction was 8.1%. Nonreportable rates for samples submitted in the first trimester vs. samples submitted in the second and third trimesters were similar, 19.4% and 19.9%, respectively. Very few non-reportable results were due to technical reasons (0.5%). The overall positivity rate for trisomy 21, 18, 13 in this cohort was <1% (n=8). Results of diagnostic testing were available for two of the eight positive cases, and both cases confirmed the cfDNA finding. Two additional cases had significant clinical findings consistent with increased risk for aneuploidy. There were no false negatives reported to the laboratory.



*There was no gestational age listed for three samples.

IV. Discussion

cfDNA screening and ultrasound represent the only options for aneuploidy screening in triplets as conventional serum screening is only available for singletons and twins. In this triplet cohort the relatively low positivity rate (<1%) could be explained, in part, to an increased rate of IVF conceptions with normal preimplantation genetic testing for aneuploidy (PGT-A). Higher nonreportable rates, than generally seen in cfDNA, are largely attributed to more stringent fetal fraction requirements in multifetal pregnancies. Example of this is seen in the 8.1% average fetal fraction of nonreportable samples, which normally would be well within the reportable range for a singleton or even twin pregnancy, however the fetal fraction requirements for triplets are approximately three fold. Nonreportable rates were consistent across trimesters in this cohort. Therefore, in triplets there is no apparent benefit in delaying screening to allow for higher fetal fractions at later gestational ages. Outcome information submitted from ordering providers suggest that cfDNA screening is an accurate and reliable screening method for triplet gestations.



*There was no indication provided on the test requisition for 12 samples.

V. References

1. Canick JA, Kloza EM, Lambert-Messerlian GM, Haddow JE, Ehrich M, van den Boom D, et al. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. *Prenat Diagn* 2012; 32:730–4.
2. American College of Obstetricians and Gynecologists. ACOG practice bulletin no.144: multifetal gestations: twin, triplet, and higher-order multifetal pregnancies. *Obstet Gynecol* 2014; 123(5):1118–32.
3. Jensen TJ, Zwiefelhofer T, Tim RC, et al. High-throughput massively parallelsequencing for fetal aneuploidy detection from maternal plasma. *PLoS One* 2013; 8(3):e57381. doi: 10.1371/journal.pone.0057381. Epub 2013 Mar 6.
4. Zhao C, et al. Detection of Fetal Subchromosomal Abnormalities by Sequencing Circulating Cell-Free DNA from Maternal Plasma; *Clin Chem*. 2015 Feb 20.

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Poster presented at American Society of Human Genetics (ASHG)'s 69th Annual Meeting; 2019 October 15-19; Houston, TX.