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ORIGINAL ARTICLE

Measuring fetal brain and lung transcripts in amniotic fluid supernatant: a comparison of digital PCR and RT-qPCR methods

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ABSTRACT

Purpose: Amniotic fluid (AF) cell-free RNA is a promising source of information regarding fetal physiology. Digital PCR (dPCR) is a direct approach to nucleic acid detection that reports absolute transcript copy number. The aim of this study was to compare quantification of cell-free fetal brain and lung RNA transcripts in AF by reverse transcription-qPCR (RT-qPCR) and dPCR.

Material and methods: Prospective hospital-based study was performed in 2016–2017. Pulmonary genes were quantified in term AF samples collected at elective cesarean birth; neurodevelopmental genes were measured in preterm samples (<34 weeks) obtained from women undergoing clinically-indicated amniocentesis.

Results: All 11 women in the term cohort had three lung transcripts and a reference gene successfully amplified from their AF supernatant using RT-qPCR and dPCR. SFTPC was the most abundant lung transcript, present in higher concentrations than the reference gene in seven of the eleven samples. Neurodevelopmental gene transcripts in 12 preterm pregnancies were less reliably detected by both methods and were present in low copy numbers (<10 copies/μl). We observed significant positive correlations between transcript quantification by RT-qPCR and dPCR.

Conclusion: This study confirms the presence of several potential mRNA markers of lung and brain development with dPCR and RT-qPCR, and a high correlation between the two methods. Transcripts of presumed fetal brain origin are present in very low copy numbers, which presents challenges to their feasibility as biomarkers of neurodevelopment.

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