Rachel Scott – Latham Award
Request to spend remaining funds on additional test and extend the period of performance

To test our hypothesis that placentas exposed to HIV age at an accelerated rate, we proposed a retrospective matched cohort with secondary analysis of tissue-banked, formalin fixed paraffin embedded placentas of singleton pregnancies of women with HIV. We performed methylation studies on HIV-exposed placentas and healthy controls to evaluate differences in epigenetic age between the two groups. Additionally, we investigated differences in inflammatory markers known to contribute to tissue aging and histopathology differences between groups. The overarching goal was to establish feasibility and collect preliminary data as the requisite next step to applying for funding to study, better understand, and eventually diagnose, predict and prevent placental pathophysiology related to HIV.

Methylation studies: We performed methylation studies on HIV-exposed placentas and control placentas but did not find differences in epigenetic age between the two groups. It is unclear if this is due to the samples being formalin-fixed, paraffin-embedded (FFPE) samples, compared to fresh, frozen samples.

Inflammatory marker studies: Due to concern for low RNA quality and quantity from the placental FFPE samples,11 out of 16 samples were used for “library prep” after RNA extraction. 6 out of 11 samples showed better library quality and quantity with slight or no gDNA contamination. After pooling and concentration by Speed Vac, only two samples were completed (FFPE A003 and FFPE B001) and passed the QC criteria to be deemed ready for RNASeq. We concluded that the RNASeq is not an effective approach to study placental FFPE samples. We then used data from the MethylSeq performed above to select a list of "genes of interest" in order to perform a targeted qRT-PCR. Unfortunately, due to sample quality, this was again not successful.

We believe this issue largely lies in the poor quality of the FFPE placental tissue. Given that DNA is more stable than RNA, we are requesting an extension of the grant period and to spending the remaining funds to complete a targeted DNA analysis of genes of interest.