

Lab methods

The oligonucleotide PCR primers (strands of nucleic acid for DNA synthesis from RNA) used were:

H3f3b, forward 5' - TGCAGGAGGCTAGCGAAGC-3', reverse 5'-

GGCGAGCCAACTGGATGTC-3' and for *Gapdh*, forward 5'-

GGTATCGTGGAAGGACTCATG-3', reverse 5'- GGATGATGTTCTGGAGAGCC-3'.

The reactions were performed using SYBR Green I chemistry (two step qPCR Master Mix, Eurogentech) with final concentrations of 0.3 μ M for *H3f3b* primers and 0.1 μ M for *Gapdh* primers, respectively. For each time point cDNA was diluted 1:5 and amplified using the following PCR conditions: 2 minutes at 50⁰C (as per Eurogentech protocol) followed by 10 minutes at 95⁰C, then 95⁰C for 15 seconds and 60⁰C for 1 minute for 49 cycles. Each sample was amplified in triplicate and the *H3f3b* was then normalised to *Gapdh* expression.

As metrics of goodness of fit of the data to a sinusoidal pattern we used the estimated amplitude for any given infant at any given week and the 'R squared' statistic (which may be interpreted as the proportion of total variance in the observed data that is explained by the fitting pattern). Samples were selected and analysed from oldest age of each participant until the earliest age at which no rhythm seen.

When making comparisons, the mean for each infant was calculated and these were compared using the t test to take into account repeated measures. Linear regression growth curve modelling was performed to determine the magnitude of change with infants' age for hormones and gene expression. Random effects were included to take into account the repeated measurements.