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COMUNICAÇÃO ORAL 3

Profiling of rat mRNA transcripts degradation for the postmortem interval estimation

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Resumo

Introduction: The accurate establishment of the period between the moment of death and the moment that a cadaver is found (postmortem interval, PMI) is one of the greatest challenges of forensic pathology. As of today, this key information in criminal investigations essentially relies on inaccurate observational methodologies like algor, livor and rigor mortis. Several studies showing the value of molecular and genetic techniques have been published but we still lack a thorough analysis of genes in different biological matrixes in a single study in order to better understand which path is the most reliable. **Aim:** The present work aims to improve the established techniques in forensic sciences to determine the PMI by providing a robust mathematical model using reverse transcription qPCR methodologies. **Material and Methods:** 66 adult male Wistan Han rats were euthanized by CO2 inhalation and left to decompose in a controlled environment (20 ºC) for 0, 3, 6, 9, 12, 18, 24, 36, 48, 60 and 72 hours. Total blood, prostate, kidneys, liver, cardiac muscle, quadriceps femoris muscle, bone marrow and right eye were sampled (approximately 25 mg) and immediately stored in 500 µL RNA later solution at -80 ºC. All specimens were homogenized by vigorous and uniform shaking with 2.8 mm ceramic beads in a OmniBead Ruptor 12 equipment. mRNA was then isolated from each sample and concentration was assessed in a Nanodrop Spectrophotometer equipment. cDNA from all samples was synthesized from diluted mRNA (250 ng) and qPCR with SYBR Green detection was performed. A couple of ubiquitous transcripts (Psmd11, Vps29) and the most expressed tissue-specific housekeeping genes for each target tissue under analysis (Hebp2, Gusb, Pdap1, Nop10, Rpl41, Rplp0, Rps27 and Rps29) were selected. **Results:** Nop10, Rpl41, Rps27, Rps29 and Vps29 genes appear to be expressed in all the tested biological matrixes. Ct values of each sample from each organ were pooled against time (PMI) in order to better understand the correlation between them. The overall procedure is time-efficient and provides predictive values in the scope of this study. **Conclusion:** The obtained results suggest that this work will produce important insights in PMI estimation, replicable in human samples. The successful development of this work might allow the translation of this formula into a daily application.

Keywords: *postmortem* interval; Wistar Han rats; housekeeping genes; mRNA transcripts; qPCR.

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