

ASSESSMENT OF PROCEDURAL ASPECTS AND QUALITY CONTROL IN HUMAN PLACENTAL RNA ISOLATION PROTOCOLS

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High quality RNA is of paramount importance in accurately interpreting gene expression changes in the placenta throughout pregnancy, as well as in common placental pathologies. The purpose of this study was to develop a standard operating procedure for the collection of human placental tissue and isolation of high quality RNA for pregnancy-related molecular studies. To accomplish this task, we compared several different parameters to minimize RNA degradation, including preservation (liquid nitrogen vs. RNeasy), disruption (mortar/pestle vs. homogenization), and isolation (Trizol vs. RNeasy). We performed 150 RNA isolations from 30 term placentas. The overall yield was 365 ± 197 ng RNA per mg of tissue. The A260/280 ratio for all samples was 2.11 ± 0.1 (mean \pm s.d.) and the RQI was 7.1 ± 1.4 . No significant differences in RNA purity, yield, or quality were observed between different placental collections or RNA isolation techniques. However, poor RQI values of 2.7 to 3.3 were obtained after brief thawing of frozen placental samples. We also compared storage of RNeasy stabilized tissue at 4 degrees or room temperature for 1 day, 7 days, and 30 days. The integrity of RNA stored at room temperature for 1 day was significantly better ($P < 0.05$, RQI 7.3 ± 0.58 , mean \pm s.d.) than RNA stored at room temperature for 30 days (RQI 5.0 ± 1.2 , mean \pm s.d.). The results of these studies will be useful for establishing standard procedures for placenta collection for pregnancy biobanks.