

OP-23: Developing a protocol for RNA extraction and quantification from amniotic epithelial cells in gestational diabetes mellitus (GDM) and non-GDM mothers

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Introduction: Human amniotic epithelial cells (hAECs) are a source of perinatal stem cells. The altered environment in gestational diabetes mellitus (GDM) is known to leave genomic imprints on hAECs which are potential biomarkers of GDM. Direct extraction of RNA from hAECs; instead from culture-expanded hAECs; would provide better quality RNA for further studies. We aimed to develop a protocol for direct RNA extraction using PAXgene® microRNA kit from hAECs and comparative study of RNA amount extracted from GDM versus a non-GDM cohort.

Methods: In keeping with the principles of Declaration of Helsinki; following ethical approval (ERC No: 21-014) and institutional approvals; gravid females undergoing caesarean section deliveries were recruited from De Soysa Hospital for Women. The amniotic membranes (AM) of GDM (n=7) and non-GDM (n=10) placentae were subjected to the following RNA extraction protocols. Protocol-I: Followed all the steps as defined by the PAXgene® microRNA kit manufacturer including embedding in wax and tissue sectioning prior to RNA extraction steps. Protocol-II: The AM were kept in a stabilising solution and directly subjected to RNA extraction steps bypassing the wax embedding step. The quality of RNA extraction was checked by using gel electrophoresis and quantified using a Qubit-3-fluorometer. Independent sample t test was used to compare the two groups.

Results: The GDM and non-GDM population mean ages were 33.85 and 30.7 years. RNA extraction results using Protocol-I- RNA detection using gel electrophoresis, none for both groups (GDM n=7; non-GDM n=10). Protocol-II- RNA was detected by gel electrophoresis as single bands; the mean RNA quantity for GDM (n=7) = 23,177.14 ng/ml (SD=32,189.88) and non-GDM (n=10) = 34,345 ng/ml (SD=40,339.29) (p=0.536).

Conclusions: A protocol for RNA extraction using PAXgene® microRNA kit was developed. Greater RNA extraction yield using protocol II needs to be verified in a repeat study. Whether the lower RNA yield from the GDM group is disease related, requires verification using a larger cohort.

Keywords: gestational diabetes mellitus, amniotic membrane, RNA extraction, PAXgene® microRNA kit