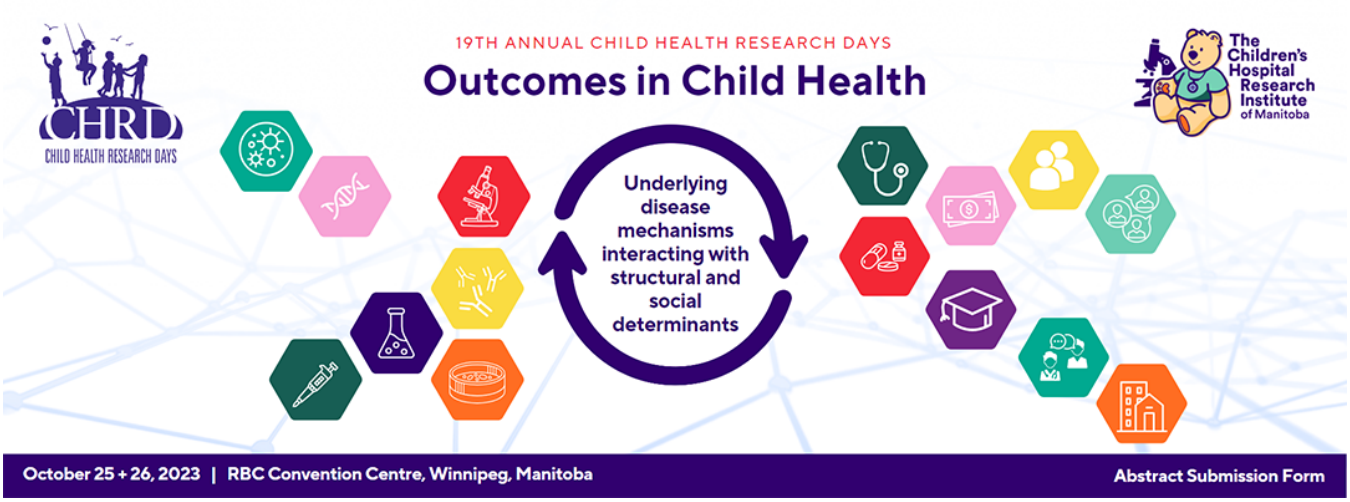

CHRD 2023: Abstract Submission Form

Submitter Name
Aya Tuma

Presenter Name
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Presenter Status
Undergraduate Students

Research Category
Basic Science

Role in the project
Perform Experiments
Analyze Data
Write Abstract

Title
Establishing a Cre-LoxP reporter mouse model to track fetal extracellular vesicles in maternal tissues

Background
Extracellular vesicles (EVs), nanoparticles involved in cellular communication, are promising biomarkers for diagnosing congenital disorders through detection of fetal-EVs (fEVs) in maternal blood.

Objective
To generate proof-of-concept, we used a cyclic recombinase (Cre)-LoxP reporter mouse model to track fEVs across the placenta and in maternal blood during gestation.

Methods
C57BL/6 Vasa-Cre positive sires, that express Cre mid-gestation in gonads, were bred with Ai14 dams expressing a floxed tdRed gene. Ai14s were sacrificed as: non-pregnant (NP, N=6) and at gestational days (GDs) E12.5-13.5 (N=2), E15.5-E16.5 (N=3), and E17.5-18.5 (N=7). Maternal and fetal tissues were imaged for tdRed expression using in vivo imaging system (IVIS) and immunofluorescence. EVs in maternal plasma (50µL) were isolated using size exclusion chromatography and characterized using tunable resistive pulse sensing. Data were analyzed using one-way ANOVAs and Tukey's post-hoc tests.

Results
IVIS imaging showed tdRed expression in Cre/Ai14 fetuses at all GDs likely due to recombination at fertilization. Both fetal layers (labyrinth, chorionic plate) and fetal-maternal zones (junctional, decidual) were tdRed-positive. There was an incremental increase in tdRed in decidual zones through GDs (E13.5 to E17.5) indicative of fEVs trafficking Cre from the fetus. NP mice were tdRed-negative. There was no

significant difference in average EV size or stability during GDs. Raw EV concentration [F(3,14)=3.50, p=0.0441] fluctuated during GDs and decreased 0.210-fold on E17.5-18.5 vs. E15.5-16.5 (p=0.0338). Normalized EV concentration to number of pups [F(2,9)=4.155, p=0.0527], showed 4.527-fold increase on E15.5-16.5 vs. E12.5-13.5 (p=0.1237) and a 0.267-fold decrease on E17.5-18.5 vs. E15.5-16.5 (p=0.054).

Conclusion
Incremental tdRed expression in maternal layers of placenta with gestation is indicative of fEV trafficking. EV concentration appears to increase during early gestation, but declines by E17.5-E18.5. Using fetal-specific proteins (e.g., syncitin) to trace fEVs in maternal tissues/plasma will provide corroborating evidence to support the premise of using fEVs for diagnosing congenital disorders.

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