Postpartum Plasma-Derived Exosomes Confer Mitochondrial Stabilization and Neuroprotection against Ischemic Stroke

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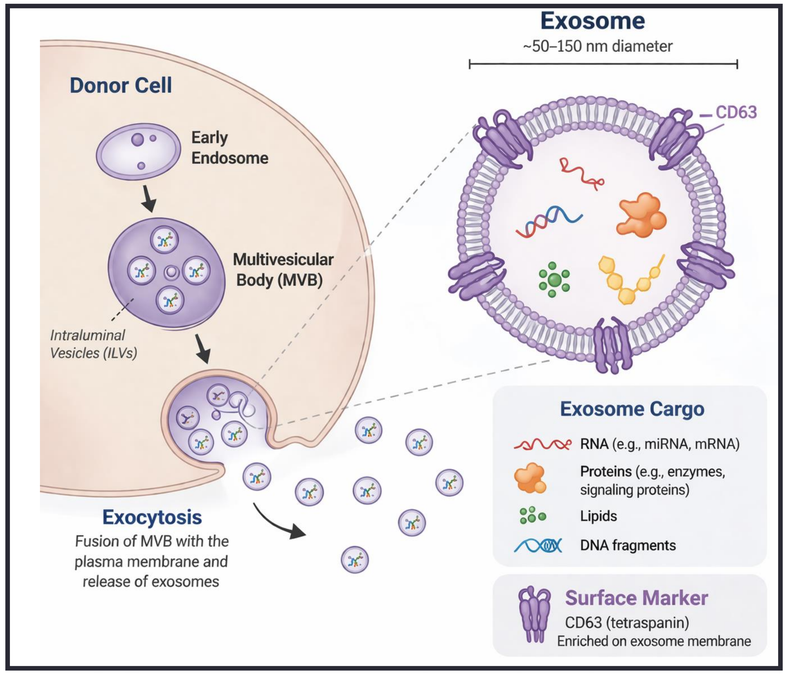
NO DISCLOSURES

BACKGROUND AND HYPOTHESIS

- The immediate (2 day) post-partum period is associated with **~9-fold higher risk of cerebral vascular accident (CVA)** compared to overall stroke risk in young adults.
- However, our prior work (1) in mice demonstrated that the postpartum period in parallel affords robust protection against experimental CVA.
- This suggests that adaptive changes occur in the immediate post-partum period to provide additional **neuroprotection** *in the event of maternal stroke*.
- **Exosomes** are secreted extracellular vesicles that serve as intercellular signaling moieties determined by their cargoes.
- We hypothesized that circulating exosomes contain bioactive cargo (such as **Hsp20**) that confer neuroprotection against ischemic injury during the postpartum period.



STUDY DESIGN AND METHODS

1. Exosomes (Exos) were isolated from plasma of postpartum female mice (**ppExos**) and from age-matched nulliparous female mice (**conExos**).

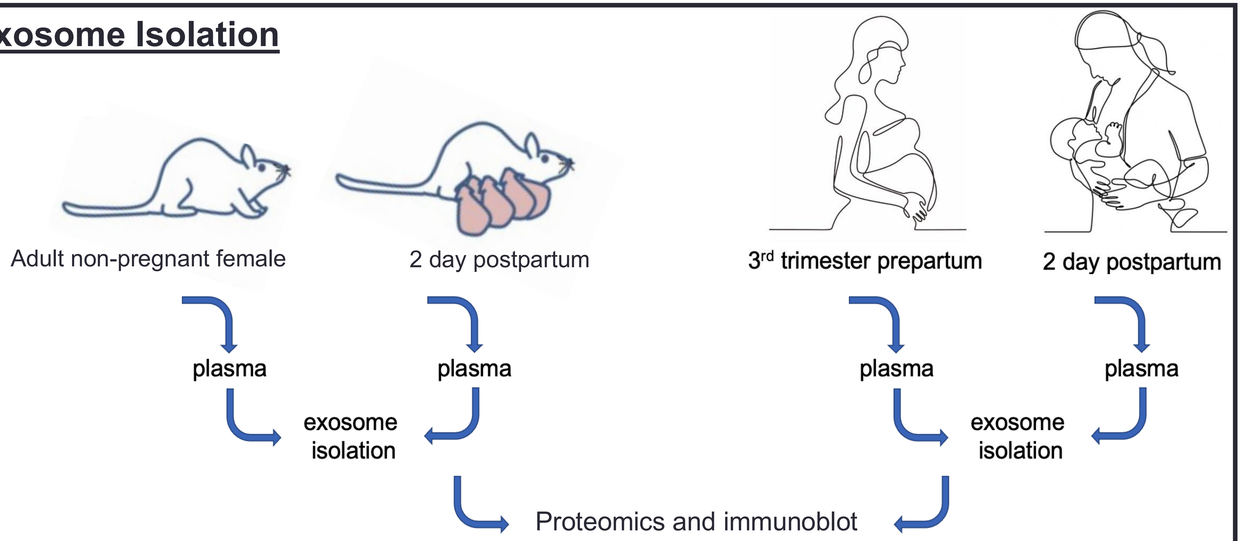
2. In parallel experiments, exosomes were isolated from serial blood samples obtained from healthy human volunteers during pregnancy (3rd trimester) and again on postpartum day 2.

3. Exos proteins were interrogated by proteomics and validated by immunoblot.

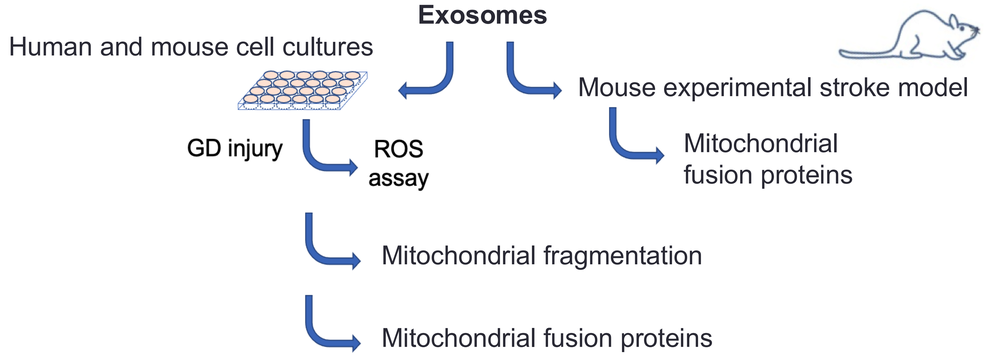
4. Protection with exosomes was assessed *in vitro* using mouse neuronal and astrocyte cultures and human RPE cell line. Reactive oxygen species (**ROS**), mitochondrial fragmentation and mitochondrial fusion proteins (**Mfn2, Opa1**) were measured after Exos treatment and simulated ischemia.

5. Neuroprotection *in vivo* was assessed by measuring infarct volume and neurobehavioral scores 24h after 1h transient middle cerebral artery occlusion (**MCAO**) with 2hr post-injury Exos intravenous treatment.

Exosome Isolation



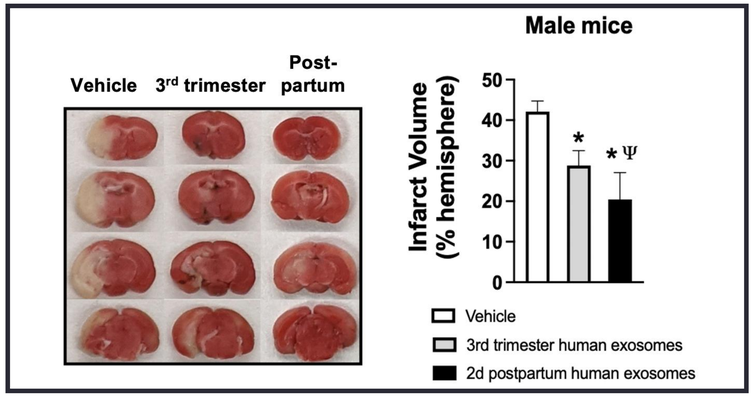
Experimental Workflow



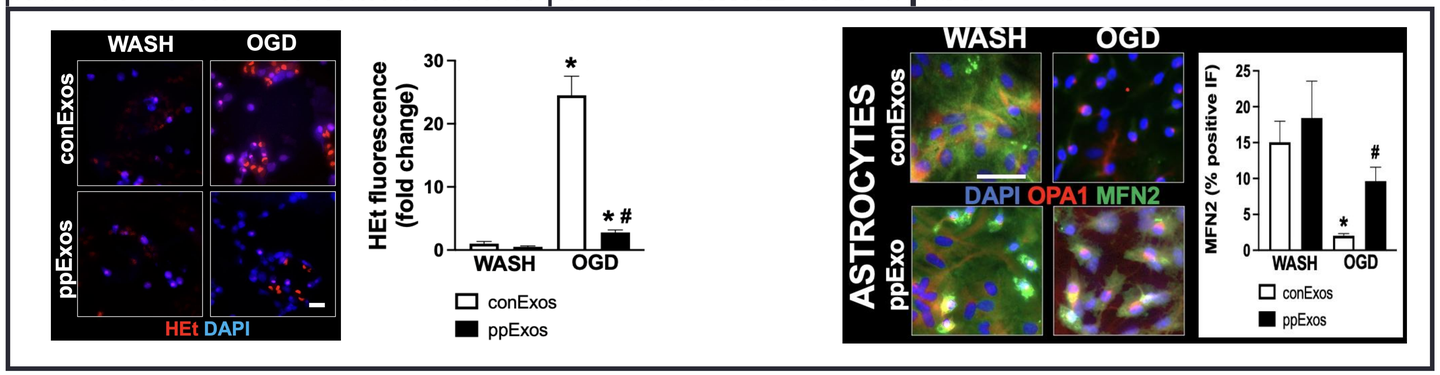
RESULTS

1. Mouse ppExos **reduced infarct volumes** and improved neurological deficits in both young adult males and females, and in aged female mice.

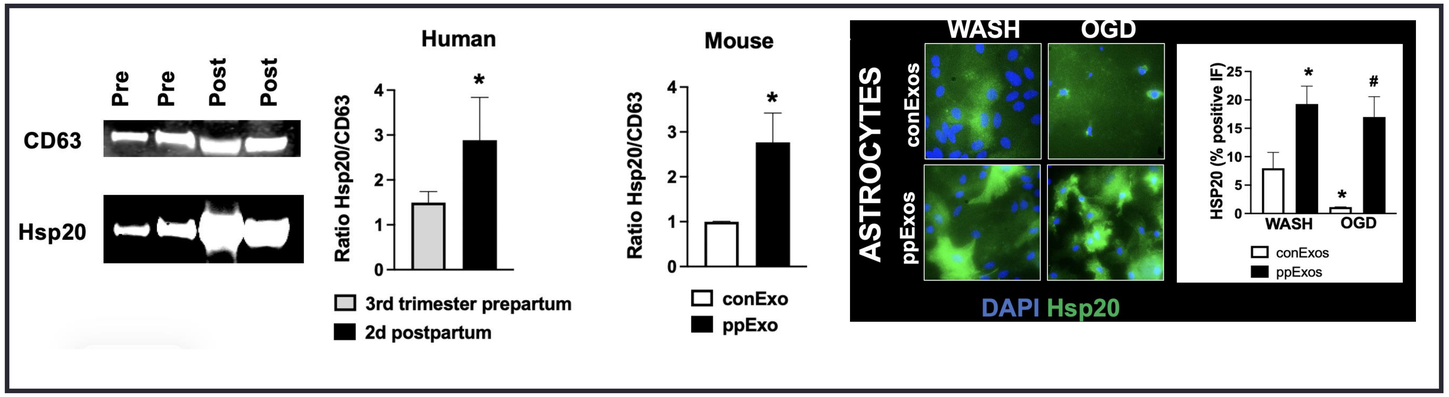
2. Human 3rd trimester and 2d post-partum Exos significantly **reduced infarct volume** and improved neurological deficits in male mice.



3. *In vitro*, both ppExos and 2d postpartum Exos **reduced ROS generation** in all cell types after simulated ischemia, **reduced mitochondrial fragmentation** and preserved mitochondrial fusion proteins **Mfn2 and Opa1**.

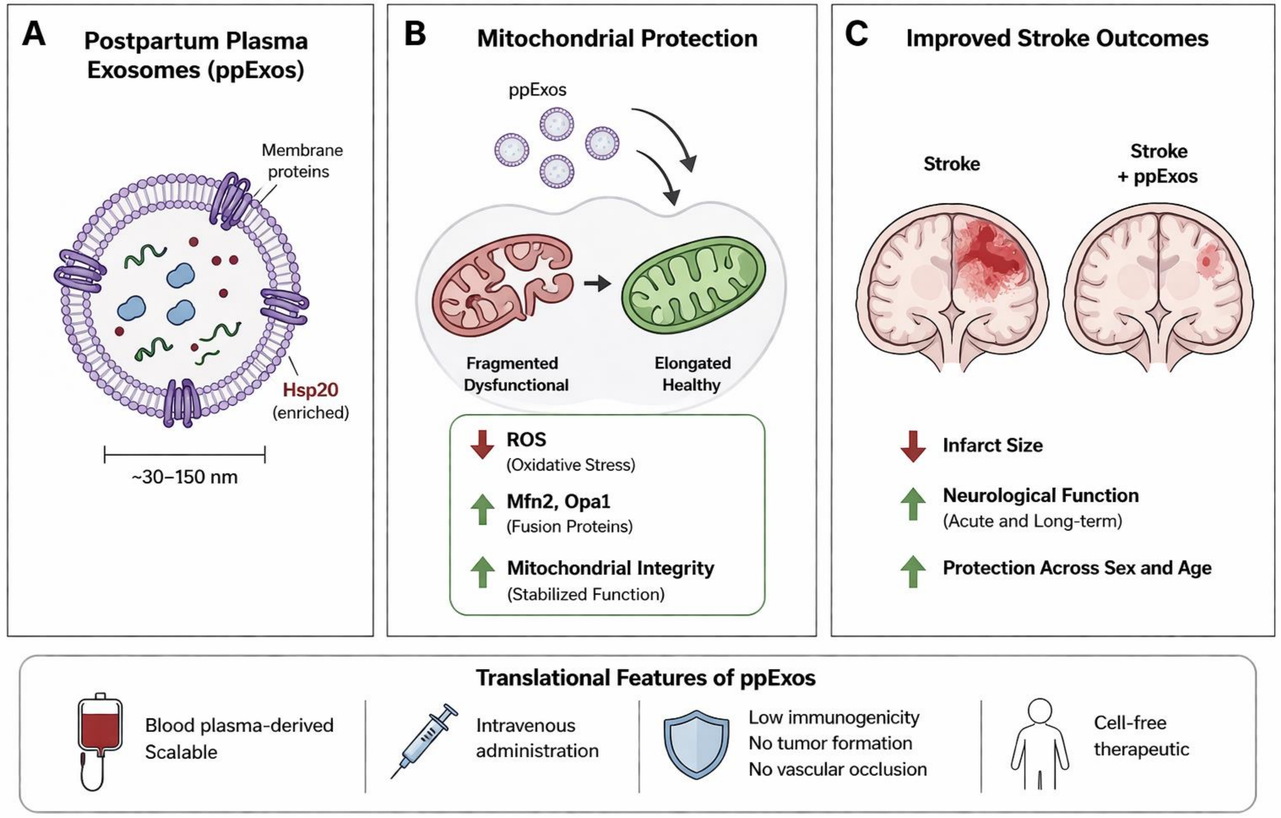


4. Heat shock protein 20 (**Hsp20**) was relatively elevated in both human 2d post-partum Exos and mouse ppExos. Simulated ischemia significantly reduced Hsp20 levels in astrocyte and neuronal cultures, **an effect reversed by ppExos**.



CONCLUSION AND DISCUSSION

- Our study identifies ppExos as **potent** and **naturally occurring** agents of neuroprotection against CVA.
- These findings position postpartum exosomes as a biologically tuned, sex-informed, and cell-free therapeutic platform with high translational potential for stroke therapy.
- Exosomes can be isolated from blood plasma, **scaled** for clinical use, and administered intravenously. Unlike live cell therapies, exosomes **pose fewer risks** of immunogenicity, tumor formation, or vascular occlusion.
- Furthermore, the postpartum period may serve as a model for other condition-specific states of enhanced repair.
- Future efforts should focus on identifying the cellular origin of ppExos and determining whether their production can be induced or engineered in non-postpartum donors.



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