Pregnancy Increases Platelet Reactivity and Induces a Thrombogenic Platelet Transcriptome in Mice

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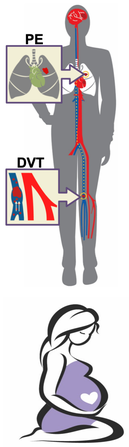
Introduction



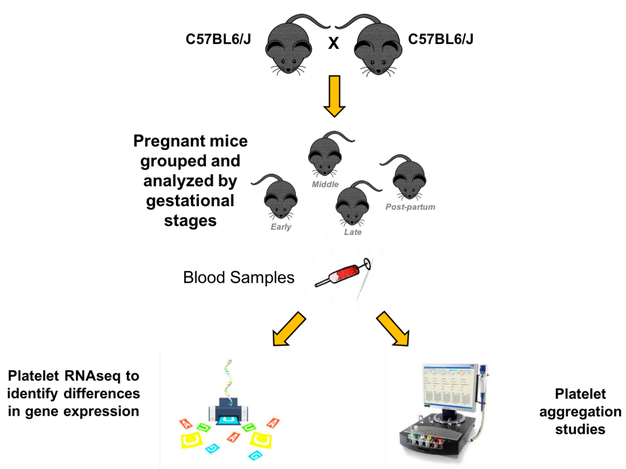
- Pregnancy is a hypercoagulable state
- Venous thromboembolisms (VTE) is a leading cause of maternal deaths[1,2]
  - 3% of maternal deaths world-wide[2]
  - 15% of maternal deaths in the United States[2]
- Platelets are derived from megakaryocytes and have a half-life of 5-10 days
- Activated platelets deliver clotting proteins directly to the blood clot
- Platelets contain a spliceosome that processes pre-mRNA allowing them to respond to external stimuli[3,4]
- Pathophysiological states can alter platelets and their transcriptomes[5,6,7]

Aims and Objectives

Aim I: Perform comparative transcriptomic analyses of platelets from pregnant mice at 4 stages of pregnancy.

Aim II: Perform functional studies on platelets from pregnant mice at 4 stages of pregnancy.

Methods



Study Design. C57BL6/J mice were bred to generate pregnant mice. The pregnant mice were divided into groups based on gestational stages: early (7-9 days post coitus), middle (11-14 days post coitus), late (16-20 days post coitus), or postpartum. Blood samples were collected and used for platelet aggregation studies to assess for functionality changes or platelet RNA sequencing to identify differences in gene expression. Non-pregnant C57BL6/J mice were used as negative controls.

Results

Platelet Aggregation in Mice Increased in the Late Gestational Group

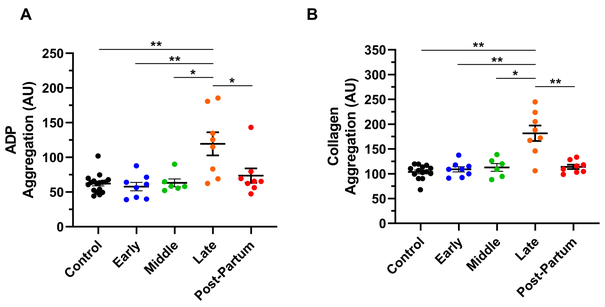


Figure 1: Platelet aggregation studies with (A) ADP as the platelet agonist and (B) collagen as the platelet agonist.

Platelet Aggregation Area Under the Curve Increased in the Late Gestational Group

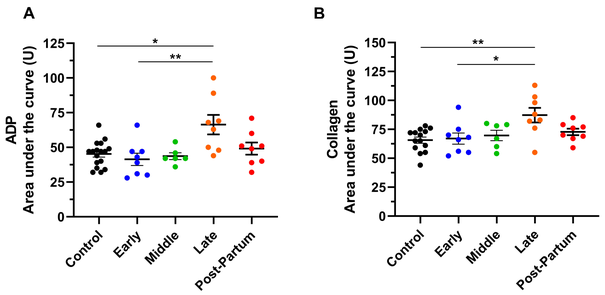


Figure 2: Area under the curve with (C) ADP as the platelet agonist and (D) collagen as the platelet agonist.

Velocity of Platelet Aggregation Increased in the Late Gestational Group

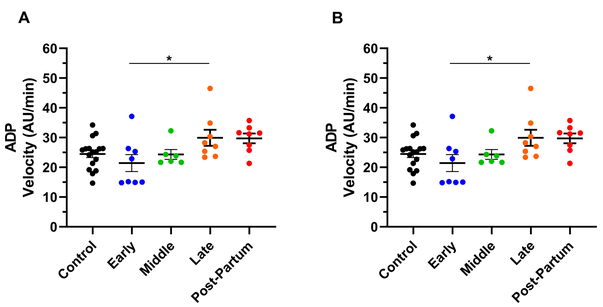


Figure 3: Velocity of platelet aggregation during the course of pregnancy and immediate post-partum period with (A) ADP as the platelet agonist and (B) collagen as the platelet agonist.

Results

Blood Volume and Platelet Count Changes are Consistent with Changes Observed in Humans

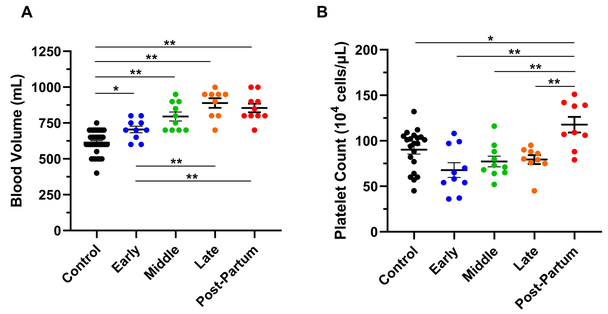


Figure 4: (A) Blood volume and (B) platelet count changes during pregnancy and immediate post-partum period.

Blood Analysis Identifies Differences in Multiple Parameters Between Groups

	Control Ave	Early Ave	Middle Ave	Late Ave	Post-Partum Ave	Control vs Early P-value	Control vs Middle P-value	Control vs Late P-value	Control vs Post-Partum P-value
WBC	0.1971	0.2090	0.185	0.1544	0.1422	0.673	0.6226	0.018***	0.007****
abs_lymphs	0.1632	0.1620	0.148	0.1100	0.1000	0.965	0.4905	0.002***	0.000****
PLT	82.9118	67.8000	77.2	79.3333	117.6667	0.122	0.442	0.589	0.003****
MPM	1.2968	1.3410	1.346	1.3789	1.3500	0.002*	0.001**	0.004***	0.005****
MPC	22.8618	25.3400	24.46	23.9444	24.0778	0.009*	0.017**	0.191	0.015****
PMDW	0.4241	0.4840	0.462	0.4667	0.4678	0.013*	0.066	0.073	0.033****
HGB	0.7618	3.0200	0.86	0.9222	1.2222	0.175	0.578	0.293	0.019****
HCT	4.1470	3.7500	4.01	3.9220	5.1890	0.455	0.708	0.561	0.040****
MCH	8.2059	51.0500	10.71	12.2556	12.2111	0.146	0.123	0.001***	0.001****
MCHC	15.8676	98.4200	20.48	23.2556	23.2556	0.151	0.142	0.002***	0.001****
RDW	13.3647	13.6600	15.72	15.3333	15.5556	0.643	0.001**	0.002***	0.000****
HDW	1.8371	1.8990	2.102	1.9956	1.9556	0.499	0.001**	0.041***	0.015****

Table 1: Blood analysis. Ave: average; WBC: White blood cells; abs_lymphs: absolute lymphocyte count; PLT: platelet; MPM: mean platelet dry mass; MPC: mean platelet concentration; PMDW: platelet dry mass distribution width; HGB: hemoglobin; HCT: hematocrit; MCH: mean corpuscular concentration; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell volume distribution width; HDW: hemoglobin concentration distribution width. Analysis done with ANOVAs (n≥9).

Conclusions

- Blood volume and platelet count findings are consistent with what is observed in pregnant humans[8]
- Late gestational increase in platelet reactivity suggests platelet alterations could play a role in increased risk of VTE during pregnancy and immediate post-partum period
- Multiple blood count parameters are statistically different across gestational groups.

Future Directions

- Analyze RNAseq data and perform mechanistic studies on the gene candidates identified
- Perform studies on other strains of mice to provide clues for the impact of genetic variation
- Correlation with humans across different ethnicities
- Perform study on pregnant humans to assess for correlation and potential therapeutic treatment targets
- Perform assays to assess the impact of pregnancy on von Willebrand Factor levels

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Acknowledgements

- NIH/NHLBI
- Department of Biological Science
- American Society of Hematology

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