P10

Estrogen Exposure Inversely Regulates Von Willebrand Factor Expression and Plasma Levels in Mice.

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Background

- Von Willebrand Factor (VWF) is a procoagulant plasma glycoprotein synthesized predominantly by endothelial cells
- VWF promotes coagulation by *i)* initiating platelet activation and aggregation at sites of vessel injury and *ii)* stabilizing circulating FVIII
- Circulating VWF levels fluctuate throughout various states of female hormone fluctuation
- VWF levels increase progressively throughout pregnancy, reaching 350% of baseline levels in the third trimester (Fig. 1)[1]
- This increase is correlated with a marked surge in estrogen levels[2]

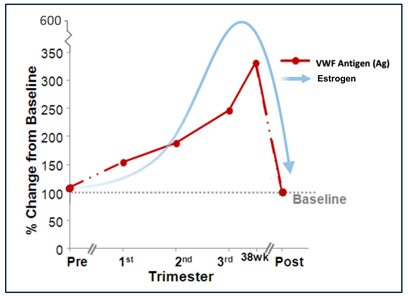


Figure 1. VWF fluctuation throughout pregnancy. Pregnancy enhances plasma VWF (red) and estrogen levels (blue) in parallel. Adapted from Drury-Stewart et al., 2014[1]

- Estrogens are a group of steroid hormones that can regulate gene transcription and activate rapid signal transduction cascades (Fig. 2)

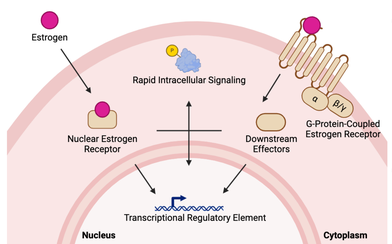


Figure 2. Estrogen Receptor Signaling Mechanisms. Estrogens bind their nuclear receptors (left) or their G-protein-coupled receptor (right) within diverse cells. Both mechanisms can work independently or together to regulate transcription and initiate rapid cell signaling.

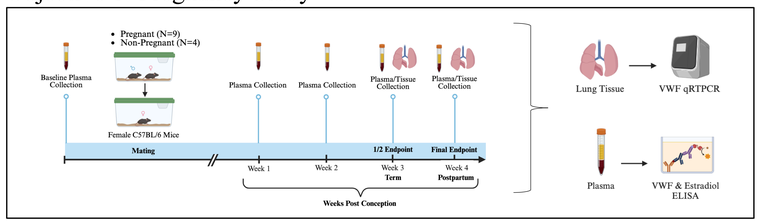
- 17β-estradiol (E2) is the most potent estrogen and the predominant estrogen in pre-menopausal women
- E2 increases in vitro endothelial cell VWF synthesis and secretion[3,4]
- The mechanisms responsible for in vivo VWF regulation by E2 are not well understood

Research Objectives

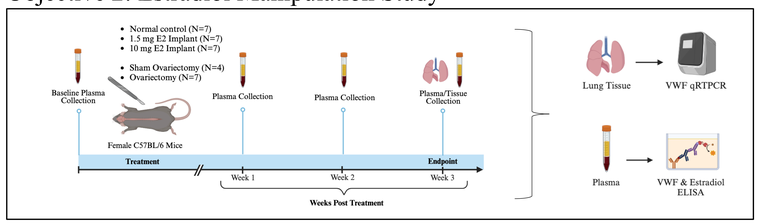
1. Verify whether the elevation of VWF in human pregnancy is recapitulated in C57BL/6 mice
2. Elucidate the underlying mechanisms by manipulating E2 levels in this mouse model

Methods

Objective 1: Pregnancy Study



Objective 2: Estradiol Manipulation Study



Results

Objective 1

VWF mRNA expression increases 1.34-fold at pregnancy term

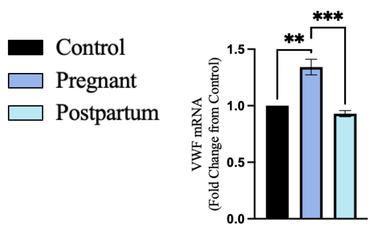


Figure 3. Endpoint lung tissue mRNA analysis. Cycle threshold (CT) numbers from pregnant (N=5) and postpartum (N=4) mice are normalized to GAPDH, expressed as fold change from control mice (N=4) and shown as mean +/- SEM. **, $p < 0.01$; ***; $p < 0.001$.

Plasma E2 increases 1.63-fold by week 2

Plasma VWF increases 3.69-fold at term

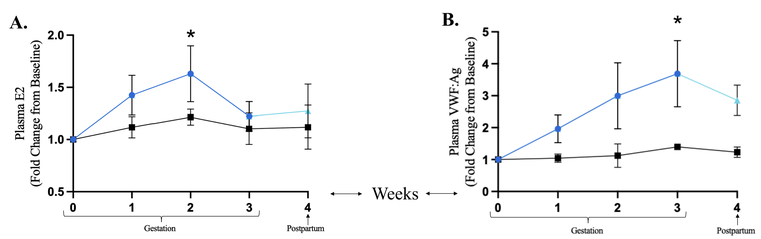


Figure 4. Plasma protein ELISA analysis. Plasma samples from pregnant (N=9), postpartum (N=5) and control (N=4) mice were analyzed by **A.** E2 ELISA and **B.** VWF ELISA. Protein levels are expressed as fold changes from baseline levels and represented as mean +/- SEM. *, significant difference from control ($p < 0.05$)..

Results

Objective 2

VWF mRNA expression dose-dependently increases with E2

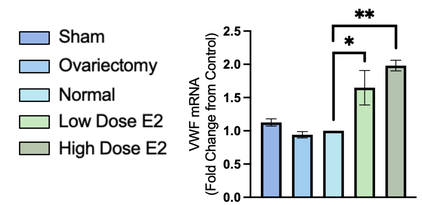


Figure 5. Endpoint lung mRNA analysis. CT numbers from ovariectomy (N=3), sham (N=2), low-dose (N=3) and high-dose (N=3) E2-treated mice are normalized to GAPDH, expressed as fold change from control (N=3) and shown as mean +/- SEM. *, $p < 0.05$; **, $p < 0.01$.

Plasma E2 dose-dependently increases

Plasma VWF **UNEXPECTEDLY** decreases

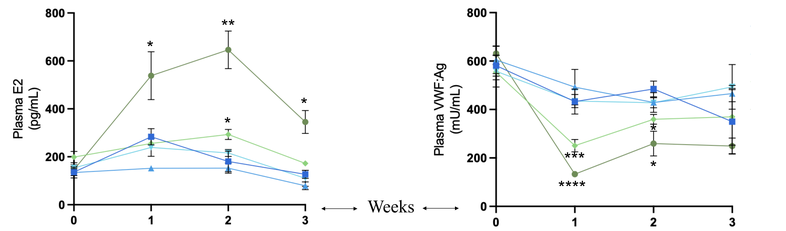


Figure 6. Plasma protein ELISA analysis. Plasma samples from ovariectomy (N=7), sham (N=4), control (N-7), low-dose (N=7) and high-dose (N=7) E2-treated mice were analyzed by **A.** E2 ELISA (pg/mL) and **B.** VWF ELISA (mU/mL). Protein levels are shown as mean +/- SEM. *; **; ***; ****, significant difference from control ($p < 0.05$; $p < 0.01$; $p < 0.001$; $p < 0.0001$).

Conclusions

- Mice, like humans, exhibit elevation of circulating VWF levels throughout pregnancy, at least in part driven by transcriptional upregulation
- Isolated increases in circulating E2 dose-dependently increase *VWF* gene transcription
- **Surprisingly**, circulating VWF levels dose-dependently decrease with E2 pellet implants, suggesting additional VWF regulation at the level of cell trafficking, release, and/or clearance

Acknowledgements

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References

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4. Kim, C. et al. Estrogen activates endothelial exocytosis. Biochem. Biophys. Res. Commun. 558, 29–35 (2021).