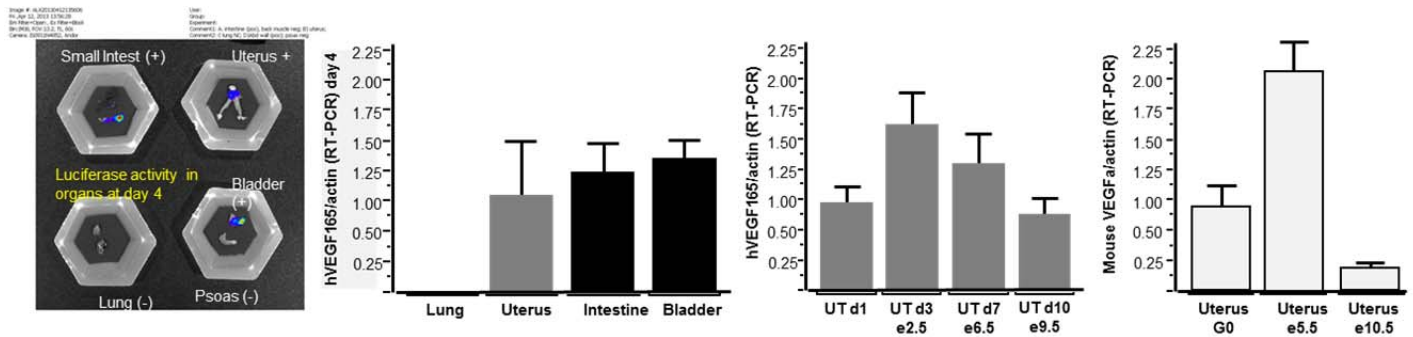


July 13, 2015

RE: **Mid-year progress report for Morgan Vision Grant 2015**

Dear Eleni and members of the medical advisory board:

Thank you again for funding our project entitled **“VEGF Gene Delivery to Uterine Spiral Arteries by Ultrasound-Mediated Cavitation of Plasmid-Conjugated Microbubbles Prevents Placental Insufficiency.”** Our objective the past 6 months has been to test whether targeted uterine vascular transfection with VEGF-conjugated microbubbles prevents abnormal spiral artery growth and abnormal uteroplacental blood flow in our transgenic model of placental insufficiency. We have transfected n=30 Tg dams (18 with VEGF and 12 with luciferase) and completed VEGF expression localization, tissue-specific expression timelines (Figure 1), uteroplacental blood flow

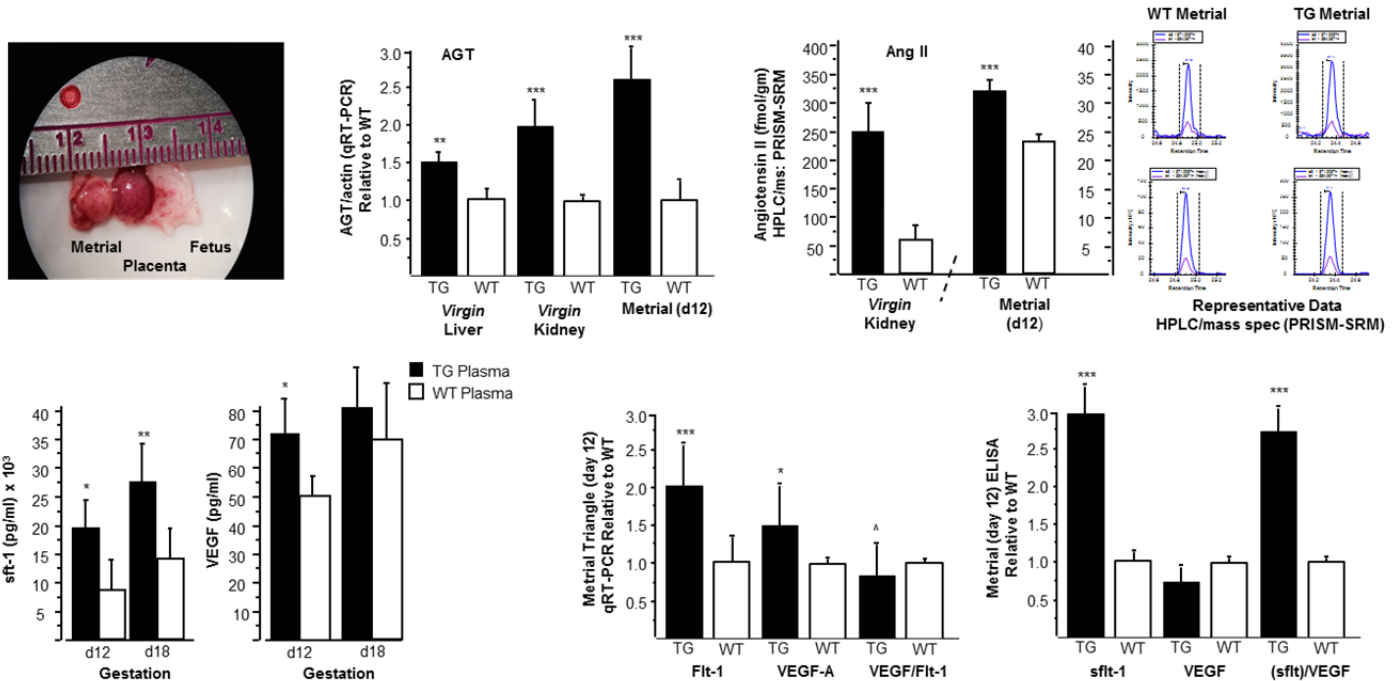


and blood volume studies using microbubble enhanced imaging in collaboration with Drs. Jonathan Lindner and Antonio Frias. Uteroplacental blood flow rate and volume are collected but not yet analyzed. We have also VEGF transfected 11 Tg dams mated with WT males and 4 WT dams mated with Tg males as of today for 3D microCT imaging. We have completed perfusion casting on all 4 WT dams and 2 of the Tg dams for imaging. We have 4 of these Tg dams currently breeding for day 16 casting and imaging and four more for metrial triangle dissections at day 12 to measure local tissue-specific VEGF expression and sflt-1 levels.

We added a new metrial triangle assay, in part because of a *JCI* paper published last year suggesting decidual tissue over-expression of VEGF leads to increased *not decreased* sflt-1 production in mice (*JCI*. 2014; 124(11): 4941-52). They did not examine the effects of decidual VEGF on spiral artery growth. We suspect decidual VEGF expression may affect pruning of the spiral arteries and perhaps nitric oxide levels (both not measured in this *JCI* paper). Both effects may cause placental damage (estimated by sflt-1 production, but not evaluated by electron microscopy, unlike our work). Therefore, to address this competing manuscript to our VEGF delivery experiment, we have added an assay of metrial triangle expression.

So far, we have completed experiments characterizing expression and protein levels in the metrial triangles of WT and Tg dams that have not been VEGF transfected for VEGF, flt-1, sflt-1, angiotensinogen and angiotensin II (Figure 2).

We measured these targets at day 12 during spiral artery angiogenesis and before placental trophoblast invasion in mice. As expected, we observe more AGT in the livers, kidneys, and metrial triangles of Tg dams compared with WT controls. Using HPLC/MS we have successfully measured angiotensin II as well, which is reproducibly increased in the metrial triangles of Tg dams. Assaying flt-1/VEGF by qRT-PCR and ELISA, we have demonstrated that Tg dams have significantly more FLT-1 expression and sflt-1 protein in the metrial triangles than WT controls.



These results not only provided a baseline for the current VEGF delivery experiments to test if VEGF delivery rescues the likely mechanism as well as spiral artery architecture, but these experiments were recommended by the editors of *JCI* to provide a mechanism that may explain why our Tg dams have less spiral artery angiogenesis than WT controls. The Preeclampsia Foundation is therefore acknowledged as partially funding our current *JCI* submission.

Our plan to complete the Preeclampsia Foundation project this year is to 1) complete analysis of uteroplacental blood flow and blood volume; 2) complete 3D microCT analysis; 3) complete analysis of metrial triangle flt-1/VEGF expression in VEGF transfected females vs controls.

We anticipate a second manuscript related to VEGF delivery and this data will be employed in our upcoming R01 submission to evaluate various “rescue” methods in our model.

Sincerely yours,

Terry K. Morgan, MD, PhD
Associate Professor of Pathology and Obstetrics & Gynecology