

## **PROJECT TITLE: FETAL CONTROL OF MATERNAL UTERINE BLOOD FLOW.**

### **Our proposal:**

There is a central role of abnormal placental blood flow in the development of preeclampsia. Placental blood flow is controlled by maternal uterine microvasculature but little is known about the control of the maternal vascular bed. We proposed to test whether the fetus can communicate its metabolic needs to the maternal microvasculature, through chemical signals, through the vasculature. We proposed to use our newly developed mouse model preparation to observe the maternal vasculature in its physiological state and test whether communicating molecules could be transferred from the fetal circulation to the maternal circulation through the intervillous space and initiate conducted responses to control the maternal vasculature. We proposed to base our experiments on our newly developed model whereby we can view gestational endometrial vasculature in situ, in a living, anesthetized mouse, using intravital fluorescence microscopy. Using this preparation we can identify and view multiple levels of the maternal vasculature including the uterine, arcuate, radial, basal and spiral artery (SA). From this visualization we can measure blood vessel diameter and therefore determine blood vessel reactivity. To determine the potential for fetal-maternal communication required further development of our preparation and required that we cannulate the fetal umbilical artery in order to introduce substances to the intervillous space and stimulate the maternal vasculature via conducted responses. Once we achieved this we had 3 specific aims to address:

**Specific Aim 1: To establish whether endometrial blood vessels can transmit “conducted responses” in the fetal-maternal direction.** Our preliminary data indicate that endometrial blood vessels have the capacity to transmit “conducted responses” in the maternal-fetal direction. We hypothesize that “conducted responses” are important in matching fetal demand with maternal blood flow, therefore, “conducted responses” must exist in the post-SA modified state in the fetal-maternal direction.

**Specific Aim 2: Determine how SA modification alters the nature of the “conducted response”.** We will compare conducted responses in the post-modified state with those in the pre-modified state.

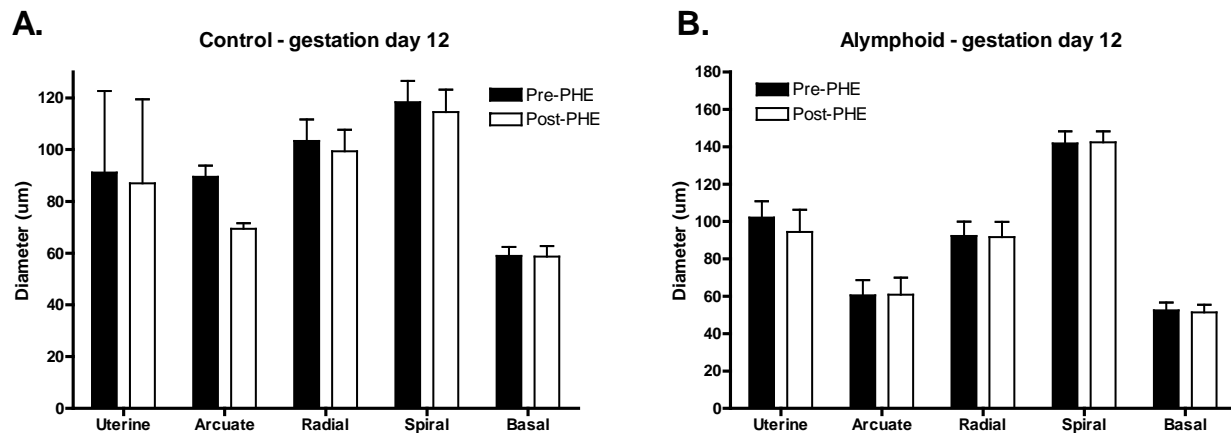
**Specific Aim 3: Investigate whether the lack of modification of the SA influences this mode of communication.** We will investigate conducted responses in the pre and post modified states in a mouse model that does not modify the SA throughout pregnancy (alymphoid mice (RAG-2<sup>-/-</sup> common cytokine receptor chain  $\gamma$ <sup>-/-</sup> ( $\gamma$ c<sup>-/-</sup>) that lack NK, B and T cells. The SAs in these animals fail to modify during pregnancy). Comparisons between control and Alymphoid mice will allow use to compare local and conducted responses through SA that do and do not modify (as in women with PE) giving us insight into the difference in blood flow regulation strategies under both conditions.

### **Results**

We tried many different methods of cannulation of the fetal umbilical artery and found that cannulation with glass micropipettes provided the most stable method of reliable cannulation and continuous perfusion. Once we focused on this technique we then worked to perfect our ability to do it reliably and repetitively.

Once perfected, we embarked on Specific Aim 1: to establish the potential for fetal-maternal communication. We used pregnant females on gestation day 12 and cannulated the umbilical artery

with  $10^{-4}$ M phenylephrine (PHE), known to induce constricted responses. We measured the diameter of different levels of the maternal uterine vasculature before and after fetal umbilical artery PHE perfusion but did not observe significant constrictions within the maternal vasculature (Figure 1A). Thus, we found no evidence of fetal-maternal communication through conducted responses. Given that specific aim 2 and 3 both centered around testing whether there was fetal-maternal communication on the unmodified maternal vasculature (specific aim 2 working with unmodified gestation day 8 and specific aim 3 working with alymphoid mice gestation day 12) we chose to next test Specific Aim 3 to avoid more technical delays that would come with learning to cannulate the gestation day 8 animals. Thus, we repeated the above experiments on alymphoid mice, gestation day 12, and, again, we measured the vascular diameter of the maternal blood vessels before and after fetal umbilical artery PHE perfusion but did not observe significant constrictions within the maternal vasculature (Figure 1B). Thus, again we found no evidence of fetal-maternal communication through conducted responses. We stopped the experiments here given our lack of evidence for fetal-maternal communication through conducted responses.



**Figure 1.** The response of different levels of the maternal vasculature to phenylephrine (PHE) perfused through the fetal umbilical artery. A) The response of different levels of the maternal vasculature to PHE perfused through the fetal umbilical artery in control animals at gestation day 12. B) The response of different levels of the maternal vasculature to PHE perfused through the fetal umbilical artery in alymphoid animals at gestation day 12.

Our data provide important evidence that the fetal-maternal direction for communication and control of the maternal vasculature through conducted responses may not be a viable route of communication. This rules out a role for conducted responses in fetal-maternal communication but questions still linger regarding the role and the importance of conducted responses in the maternal vasculature. We have evidence that conducted responses are functional in the maternal vasculature (Burke S.D. et al., *Am. J. Reprod Immunol*, 63(6):472, 2010) but do not know what role they play and how their role changes with spiral artery modification. Future research will investigate the role for conducted responses in the control of the maternal vasculature and the control of placental blood flow.

There have been no posters or publications that have come from these data as of yet.