Name: Amanda Hankes

Email: ahankes@wisc.edu

Major Professor: Dr. Ian Bird

Degree Objective: Ph.D. Endocrinology and Reproductive Physiology

Background: BS Animal Science, Univ of Illinois- Urbana Champaign

Current Research Project:

During pregnancy, the body increases its blood flow to the uterus to provide enough nutrients and oxygen to the growing fetus. Pregnancy increases endothelial cell sustained Ca2+ signaling (characterized by prolonged burst response), thereby increasing nitric oxide production instigating vasodilation of the uterine arteries to increase blood flow to the uterus. The pregnancy enhanced gap junction function has higher and more frequent sustained phase calcium bursts than non-pregnant cells and presumably in cells derived from the diseased state. This difference is essential to comparing pregnant vs. non-pregnant and healthy vs. diseased cells and helps explain the potential pathways shutting down these gap junctions *in vivo*. Failure of proper adaptation can result in hypertensive diseases of pregnancy such as preeclampsia (PE). Our lab is trying to find explanations as to why Ca2+ bursts are inhibited during PE and how to reverse this effect. It has been shown that the gap junction protein connexin 43 (CX43) is crucial to sustained phase Ca2+ responses which are inhibited by activation of c-SRC and ERK pathways. The SRC pathway has also been defined in wound healing literature to disassociate CX43 arrays through phosphorylation of the tight junction scaffolding protein ZO-1. My project will focus on the gap junction protein (CX43) interactions in healthy pregnancy and in states of stress.

Currently, I am working with Tumor Necrosis Factor-alpha (TNF), a cytokine known to be elevated in PE, and how TNF causes cell dysfunction acutely and long-term. We believe TNF works via Src (due to recovery of bursts by the Src inhibitor PP2) and ERK pathways inhibiting CX43 gap junction function. Literature suggests that TNF causes an increase in reactive oxygen species (ROS), leading to destruction of endothelial cells. However, we are trying to shine light on the possibility TNF is more damaging to cells at lower doses by inhibiting and decreasing sustained Ca2+ bursts at doses as low as 10ng/mL, a dose much lower than the 50ng/mL damaging dose that produces toxic ROS. We then take this inhibitory data and use different Src and ERK inhibitors to determine if I can rescue the damage done by TNF. Using the Ca2+ dye Fura-2 AM, I image and analyze the sustained [Ca2+]i bursting before and after TNF exposure, with or without rescue agents. I am also using an electric cell-substrate impedance sensing (ECIS) system to measure monolayer integrity and Western blotting to measure phosphorylation sites of CX43. As a result of RNA array analysis, I am also checking different MMP inhibitors to determine if any have a recovery effect on the TNF-induced junctional protein dysfunction.

Honors & Awards:

National: Pfizer President's Presenter Award, Society of Reproductive Investigations 2016

Local at UW: Senior Speaker Award, ERP Symposium 2015

Finalist for Poster Award, ERP Symposium 2014

Poster Award, Women's Health and Health Equity Symposium 2014.

Grants Received: Currently funded under NIH PO1 HD38843.

Research previously funded under NIH T32 Trainee; NIH Ruth L. Kirschstein National Research Service Award NIH T32-HD041921 2014-2016.

Publications:

Boeldt DS, **Hankes AC**, Alvarez R, Nauman K, Balistreri M, Grummer MA, Yi FX and Bird IM. (2014). Pregnancy Programming and Preeclampsia: Identifying a Human Endothelial Model to Study Pregnancy-Adapted Endothelial Function and Endothelial Adaptive Failure In Preeclamptic Subjects. *Proceedings of the 40th Anniversary of the Center for Perinatal Biology. Springer*. pp27-47.

To be Submitted: Ampey BC, **Hankes AC**, Jobe OS, Lopez GE, Rhea K, Nguyen A, Bird IM, Magness RR. (2017). Cyclic Nucleotides Regulate Gap Junction Function and Communication in the Uterine Vasculature Endothelium during Ovine Pregnancy.

To be Submitted: **Hankes AC**, Boeldt DS, Grummer MA, Yi FX, Bird IM. (2017). Activation of Src and ERK Facilitates TNF-alpha Inhibition of Pregnancy Adapted Ca2+ Burst Function and Increased Monolayer Integrity in Ovine Uterine Artery Endothelial Cells.

National Presentations:

Hankes AC, Grummer MA, Bird IM. (2017) "Low Dose VEGF Protects, While IL-6 Amplifies TNFα-Induced Damage to Pregnancy-Derived UAEC Monolayers." *64th Annual Meeting for the Society for Reproductive Investigation.* Orlando, FL.

Ampey BC, **Hankes AC**, Bird IM, Magness RR. (2017) "cAMP Rescues the Negative Regulatory Effects of TNF-α on Endothelial Cx43 Gap Junction Function and Protects Cell Permeability." *64th Annual Meeting for the Society for Reproductive Investigation.* Orlando, FL.

Selected for Oral Presentation: **Hankes AC,** Boeldt DS, Yi FX, Grummer MA, Bird IM. (2016) TNFα Decreases Monolayer Resistance but MMP Inhibitor GM6001 Improves the Monolayer Integrity in P-UAEC. *63rd Annual Meeting for the Society for Reproductive Investigation.* Montreal, QC, Canada.

Selected For Oral Presentation: Nauman, Khurshid, **Amanda Hankes,** Derek Boeldt, Dinesh Shah, and Ian Bird. (2015) Multiple Cytokines and Growth factors associated with Preeclampsia Induce Endothelial Dysfunction in HUVECs via Src Kinase. *62nd Annual Meeting for the Society for Reproductive Investigation.* San Francisco, CA.

Selected For Oral Presentation: Nauman Khurshid, Derek Boeldt, **Amanda Hankes**, Jennifer Krupp, Dinesh Shah, Ian Bird (2014). 10:12 Conjugated linoleic acid (CLA) isomer rescues HUVEC cell dysfunction in a preeclamptic in-vitro model. *Annual Meeting for the Society of Maternal-Fetal Medicine.* New Orleans, LA.

Selected for Oral Presentation: **Hankes AC**, Yi FX, Grummer MA, Magness RR, Bird IM. (2013) TNF alpha Induces Gap Junction Dysfunction in Sheep Uterine Artery Endothelial Cells; a Model for Preeclampsia. *60th Annual Meeting for the Society for Gynecologic Investigation.* Orlando, FL.

Hankes AC, Boeldt D, Grummer MA, Magness RR, Bird IM. (2015) ECIS Monitoring Reveals TNFα and VEGF Differentially Control Sustained Loss of Endothelial Monolayer Integrity of P-UAEC. *62nd Annual Meeting for the Society for Reproductive Investigation.* San Francisco, CA.

Ampey, Bryan, **Amanda Hankes**, Ian Bird, Ronald Magness. (2015) Cyclic Nucleotide Regulation of Uterine Artery Endothelial Cell Intracellular Signaling Through Changes in Gap Junction Expression in Pregnancy. *62nd Annual Meeting for the Society for Reproductive Investigation.* San Francisco, CA.

Boeldt, Derek, **Amanda Hankes,** Ian Bird. (2015) VEGF-165 and TNF-alpha differentially regulate endothelial dysfunction in a hormone specific manner in human endothelial cells. *62nd Annual Meeting for the Society for Reproductive Investigation.* San Francisco, CA.

Hankes AC, Boeldt D, Yi FX, Grummer MA, Magness RR, and Bird IM (2014) CLA as a Src Inhibitor: An Innovative Approach to Rescue Endothelial Function in Preeclampsia. *Annual Meeting for the Society of Maternal-Fetal Medicine.* New Orleans, LA.

Michael Balistreri, Derek Boeldt, **Amanda Hankes**, Ian Bird (2014). Modeling preeclamptic endothelial failure in HUVEC-CS. *Annual Meeting for the Society of Maternal-Fetal Medicine.* New Orleans, LA.

Other Presentations:

Oral Presentation: Hankes AC. (2016) Understanding the Inhibitory Effects of TNFα on Ovine Pregnant-Derived Uterine Artery Endothelial Cells. *Endocrinology Reproductive Physiology Seminar.* Dec. 15, 2016.

Oral Presentation: Hankes AC. (2015) Long-term Effects of TNF-alpha on Monolayer Integrity and the Possible role of Matrix Metalloproteases in Uterine Artery Endothelial Cells from Pregnant Sheep. *Endocrinology Reproductive Physiology Seminar.* Oct. 29, 2015.

Oral Presentation: Hankes AC, Boeldt DS, Grummer MA, Magness RR, Bird IM (2015). ECIS Monitoring Reveals TNFa and VEGF Differentially Control Sustained Loss of Endothelial Monolayer Integrity of P-UAEC. *ERP Symposium 2015.* June 25, 2015.

Oral Presentation: Hankes AC. (2014) Inhibiting the Src Pathway to Rescue Endothelial Gap Junction Dysfunction in Preeclampsia. *Endocrinology Reproductive Physiology Seminar* Nov. 13, 2014.

Poster Presentation: **Hankes AC**, Boeldt D, Yi FX, Grummer MA, Magness RR, and Bird IM (2014) CLA as a Src Inhibitor: An Innovative Approach to Rescue Endothelial Function in Preeclampsia. Women's Health and Health Equity Symposium Sept. 18, 2014 and ERP Symposium 2014.

Oral Presentation: Hankes AC. (2013) Src Inhibitors Recover TNFα Induced Gap Junction Dysfunction in Endothelial Cells; a Potential Therapy for Preeclampsia. Endocrinology Reproductive Physiology Seminar Nov. 6, 2013.

Oral Presentation: Hankes AC, Grummer MA, Yi FX, Bird, IM (2013) TNFα inhibits sustained phase $[Ca2+]_i$ bursts via Src pathway rather than by Reactive Oxygen Species Accumulation. Endocrinology Reproductive Physiology Seminar Jan 31, 2013.

Poster Presentation: **Hankes AC**, Boeldt DS, Yi FX, Grummer MA, Magness RR, Bird IM (2013). TNF alpha Induces Gap Junction Dysfunction in Sheep Uterine Artery Endothelial Cells; a Model for Preeclampsia. WARF Discovery Challenge May 20, 2013 and ERP Symposium 2013.

Teaching and Mentorship:

- Teaching and directing other grad students in different labs to use the Electric Cell-substrate Impedance Sensing system (ECIS) and teach how to incorporate this new method to benefit their own research
- Mentored Drs. Nauman Khurshid and Michael Balistreri during their fellowship in the Bird Lab. I helped teach them cell culture and the protocol of Ca2+ Imaging on the lab's microscope and helped with experiments when needed.

Committees:

T32 Grant Student Representative: 2015- Current

Dept. Obstetrics/Gynecology Student Research Representative: 2014-Current

Student Committee Member: 2013-Current

Symposium Organizing Committee: 2013-Current

ERP Recruitment Committee: 2012-Current