

chorionic regions and are associated with HLA-G and cytokeratin-7 confirming their trophoblast identity. CDX2 cells demonstrated the potential to form a capillary network akin to endothelial cells. Placental samples from healthy (n=6) and preeclampsia (n=8) patients revealed higher levels of CDX2 expression in preeclampsia. Within preeclampsia CDX2 cells, Natriuretic peptide receptor 1 (NPR1), RET oncogene, and Homeobox D10 (HOXD10) were significantly differentially regulated, including a unique long-non-coding anti-sense RNA (KANSL1-AS1) that affected the function of CDX2 and trophoblast cells in invasion and normal vasculogenesis. **DISCUSSION/SIGNIFICANCE:** In sum, based on these observations, the present study postulates that CDX2 cells present in a healthy human placenta may serve as a prospective cellular reservoir for angiogenesis. Conversely, altered gene programs within CDX2 cells cause aberrant vascular function that could contribute to the progression of preeclampsia.

### Allogeneic Recellularized Lung Orthotopic (ARLO) Transplant Research

Douglas Tran, Dr. Douglas Tran, Dr. Bartley Griffith and Dr. Zhongjun Wu  
University of Maryland

**OBJECTIVES/GOALS:** As of 2021, the lung transplantation waiting list has a mortality rate of 7.6 deaths per 100 patient-years. Bioengineered human organs is an emerging field of tissue engineering with a goal of developing suitable organs for transplantation. The focus of the project is to evaluate the efficacy of bioengineered lungs using a human-to-swine model. **METHODS/STUDY POPULATION:** This project will involve designing and assessing the bioengineered lung by establishing a human-to-pig xenotransplantation survival model. The project aims to evaluate how well the bioengineered lung functions within a living model. The bioengineered lung is constructed using swine connective tissue scaffolding, which has been recellularized with human cells. Anatomically, the lung will resemble a swine lung but will possess the immunological and cellular markers of human tissue. The proposed model will initially assess the immunological response of swine to human lung tissue. Lung function will be assessed during surgery using pulmonary vein gas samples and tissue sampling. Following the end of the study, additional tissues samples will be taken to evaluate the immunological response to the tissue. **RESULTS/ANTICIPATED RESULTS:** Xenotransplantation and bioengineered organs are two new emerging fields of research that have just begun to enter the large animal testing phase. This model will provide a novel human-to-pig xenotransplant survival model that will be used to test the efficacy of bioengineered lungs function in a dynamic living organism. The design has taken the principles of immunology learned from the current clinical and xenotransplant research and has incorporated this knowledge into the known pig-to-pig transplant models. We anticipate that this model design will be easily reproducible and can be expanded to other bioengineered organs as an effective means to test functionality. **DISCUSSION/SIGNIFICANCE:** The COVID-19 pandemic's aftermath may lead to an increased demand for lung transplants. Bioengineered lungs could provide an additional source of organs to supplement current availability. This novel approach has the potential to offer a means to test several different types of bioengineered organs in the future.

63

### Detecting and monitoring Salmonella infection and chronic carriage in living mice using bioluminescent in vivo imaging

Aliyah Bennett<sup>1</sup> and John S. Gunn<sup>2</sup>

<sup>1</sup>The Ohio State University and <sup>2</sup>The Abigail Wexner Research Institute at Nationwide Children's Hospital

**OBJECTIVES/GOALS:** SalmonellaTyphi primarily persists in human chronic carriers by forming biofilms on gallstones in the gallbladder (GB). We developed a mouse model of GB chronic carriage, and using this model, aim to detect Salmonella in living mice and track the progression of GB carriage with bioluminescent S.Typhimurium and in vivo imaging. **METHODS/STUDY POPULATION:** S.Typhimurium 14028 (WT) was transduced with the lux operon from the S. Typhimurium Xen33 strain from Perkin Elmer®, creating 14028lux. 129X1/SvJ mice were fed a lithogenic diet for 6 weeks to induce gallstone formation. After cessation of diet, these mice were infected with 5x10<sup>3</sup>-1x10<sup>4</sup> colony forming units (CFU) of either the 14028lux isolate, WT (non-luminescent) isolate, or an equal volume of sterile saline. Mice were serially imaged (IVIS SpectrumCT) every 2-3 days for up to 63 days. Images were quantified by measuring average radiance over selected regions of interest. The presence of bioluminescent bacteria in specific organs was confirmed by imaging the abdominal cavity post-mortem. Organs were homogenized and CFUs per mg of tissue were quantified and compared between each group. **RESULTS/ANTICIPATED RESULTS:** Compared to the controls, mice infected with 14028lux showed luminescence in the abdomen as early as three days post-infection. Within 15 days, the resolution was sufficient to discriminate signal in specific organs, notably the gallbladder, liver, spleen, and cecum. The presence of bacteria was confirmed in these organs via direct imaging and by quantifying CFUs in the tissues. At 63 days post-infection, we identified >10<sup>3</sup> CFUs and significant luminescence in the GB of a portion of 14028lux-infected mice. For all days post-infection, 14028lux-infected mice that lacked observable luminescence had <100 CFUs/mg tissue. **DISCUSSION/SIGNIFICANCE:** We have developed a technique using bioluminescent S.Typhimurium and in vivo imaging that, without sacrificing infected mice, enables us to reliably distinguish between mice that have maintained gallbladder chronic carriage >60 days and those that have cleared infection.

65

### Testing the effects of rigid encapsulations on bovine primordial follicle quiescence versus growth

Kathryn L McElhinney, Erin E Rowell and Monica M Laronda

Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine

**OBJECTIVES/GOALS:** There is an interest in developing a bioprosthetic ovary for ovarian tissue transplantation. The properties of the ovarian extracellular matrix need to be better understood in order to replicate the human ovary. We tested the effects of an encapsulating hydrogel at different rigidities on bovine primordial follicle activation, growth, and survival. **METHODS/STUDY POPULATION:** Bovine primordial follicles were isolated from ovarian cortex. A mean of 9.9 follicles (range 3-24) were encapsulated per bead in either 1% or 5 % alginate across 4 experiments. The encapsulated

follicles were subsequently crosslinked in a calcium sulfate solution. Follicles were then cultured for 8 days with light microscopy imaging taken every other day along with media exchanges. Follicles were then examined using immunofluorescence. Growth and survival curves were constructed and all statistical analyses were performed using Graph Pad Prism 9. RESULTS/ANTICIPATED RESULTS: A total of 372 follicles were encapsulated across 32 beads (16 in 1% alginate and 16 in 5% alginate). There were no differences in initial follicle size between the two conditions (33.53  $\mu$ m vs. 32.45  $\mu$ m,  $p=0.47$ ). At the end of 8 days, there was no difference between follicle size (59.55 vs. 56.06,  $p=0.48$ ). Additionally, there was no difference in survival between 1% and 5% alginate encapsulation (57.75% vs. 52.43%,  $p=0.40$ ). Immunofluorescence is being performed on encapsulated follicles to confirm the presence of DDX4, a molecular marker of oocytes, after 8 days in culture. Additional encapsulated follicles have also been submitted for histologic sectioning and hematoxylin and eosin staining to better characterize the viability and health of these follicles after 8 days in culture. DISCUSSION/SIGNIFICANCE: There was no significant difference in growth or survival between primordial follicles cultured in 1% or 5% alginate gels. Immunofluorescent analysis confirmed the presence of viable follicles at the end of 8 days of culture. Future work needs to further explore how factors in the ovarian extracellular matrix impact follicle maintenance and growth.

### Predictors of Autologous Fat Grafting in Immediate, Implant-Based Breast Reconstruction

Omar Jean-Baptiste<sup>1</sup>, Owen H. Brown<sup>2</sup> and Peter W. Thompson<sup>2</sup>

<sup>1</sup>Emory University and <sup>2</sup>Emory University Plastic Surgery

OBJECTIVES/GOALS: Patients frequently need or desire fat grafting to improve common issues such as implant visibility and contour deformity, often done as a second, staged procedure following immediate reconstruction. This study aimed to identify which patient factors and reconstructive techniques predict the need for revision with AFG after IBBR METHODS/STUDY POPULATION: Patients who underwent IBBR with either tissue expanders or implants following mastectomy from 2017 to 2021 were identified. Demographics, comorbidities, and the postoperative course were reviewed. The primary outcome variable was AFG after the initial reconstruction. Univariate and regression analyses were performed to identify factors predictive of AFG. RESULTS/ANTICIPATED RESULTS: Five-hundred twenty-nine patients were included in our analysis, with 43% having AFG. Univariate regression displayed single-stage reconstruction (OR=0.53, 95% 0.37-0.75) and previous radiation (OR 0.59, 95% 0.35-0.99) negatively predicted the need for AFG, while bilateral breast reconstruction (BBR) was a predictor (OR 2.32, 95% 1.58-3.4). On multivariate analysis, decreasing age and BBR remained predictive of AFG. The odds of AFG decreased by 3% for every one-unit increase in age (95% CI [0.96, 0.99]). Interestingly, neither pre-pectoral breast reconstruction nor specimen weight:implant ratio was associated with increased need for AFG on univariate/multivariate analysis. DISCUSSION/SIGNIFICANCE: Patients requiring AFG were likely younger and had undergone BBR with tissue expanders. Plane of implant did not appear to affect need for AFG. Knowledge of these predictive factors may help plastic surgeons in preoperative counseling before implant-based breast reconstruction.

66

### Visiting endowed chair: a new model to support Hispanics junior investigators

Carlamarie Noboa Ramos, Lourdes E. Soto de Laurido and Walter R. Frontera

University of Puerto Rico Medical Sciences Campus

OBJECTIVES/GOALS: Analyze how the Endowment HIREC's Mentoring and Career Coach Model A productive mentoring relationship is essential to advance researchers into being independent and bring extramural funds. METHODS/STUDY POPULATION: Provide Hispanic researchers mentoring and career coaching to strengthen their pathway as researcher. The HiREC's Career Coach and Mentoring Component (CCMC) is an innovated approach to support long-lasting research mentoring relationships in our institution. This approach was developed to advance research to eliminate health disparities, promote multidisciplinary translational research in a Minority Institution and sustain research infrastructure and services, career, and workforce development initiatives. Promising Faculty are target and early and mid-career investigators interested in pursuing a research career. To implement the CCMC with the Visiting Endowed Chair a HiREC Advisory Leadership Group in Mentoring will be established, with researchers from Puerto Rico, and US mainland. RESULTS/ANTICIPATED RESULTS: Three Hispanic mid-career women from the School of Medicine and one from the School of Health Professions from the University of Puerto Rico received a HiREC Advanced Research Award of \$50,000. The awardees achieved their goals; completed their research plan, research infrastructure needs, peer-reviewed publications, and submission of a competitive grant. They also provided successful perspectives on mentoring relationships in a Minority institution. Each one showed the mentor's and mentee's experiences as fundamental for their research advancements, productivity, leadership, and successful results. HiREC's mentoring component with the Visiting Endowed Chairs improves a healthy work environment and expands the research agenda for each awardee sustaining the institutional research culture. DISCUSSION/SIGNIFICANCE: A productive mentoring relationship is essential to advance researchers into being independent and bring extramural funds. Four mentees received formal, long-term guidance and endowment funds for their research infrastructure requirements with successful outcomes. HiREC contributes to building up an institutional mentoring program.

72

### Participant Recruitment at OHSU: Equipping Researchers to Overcome Recruitment Challenges

Meredith Zauflik, Kitt Swartz, Cynthia D. Morris and David H. Ellison

Oregon Health & Science University

OBJECTIVES/GOALS: Under enrollment of trials is a continued challenge in clinical research. In response, the Oregon Clinical and Translational Research Institute (OCTRI), the CTSA at Oregon Health & Science University (OHSU), launched a central resource, OCTRI Recruitment, to equip researchers with the knowledge and tools needed for recruitment success. METHODS/STUDY POPULATION: OCTRI Recruitment focused programmatic development in response to the voice of OHSU researchers. In 2018, a