TABLE OF CONTENTS

2 Welcome to the Large Animal Genetic Engineering Summit

3 Program

6 Keynote Lecture

7 Session 1: Gene Editing Technology in Livestock Applications

10 Session 2: Modification of Disease Resistance and Susceptibility in Large Animals

13 Session 3: Transgenic Large Animal Models

17 Session 4: Regulation of Genetically Engineered Large Animal Models and Medical Device Development Tools

19 Session 5: Current and Future Large Animal Models in Biomedical Research

22 Session 6: Funding Mechanisms to Support Genetically Engineered Livestock Research

24 Session 7: Current and Future Large Animal Models

25 Poster Abstracts

Index

LARGE ANIMAL GENETIC ENGINEERING SUMMIT ORGANIZERS
Utah State University College of Agriculture and Applied Sciences

**LAGE Summit Committee**
Chris Davies, Co-chair
DeeVon Bailey
Irina Polejaeva, Co-chair
Lynnette Harris
Tammy Firth

**Assistants**
Aaron Thomas
KailCee Harrison
Kenneth Olsen
Kirk Salisbury
Michael Wernert
Shelby Ruud
Terence Larson
Welcome from the Summit Co-Chairs

Dear Colleagues and Friends,

It is our great pleasure to welcome you to the first Large Animal Genetic Engineering (LAGE) Summit. Our objectives for the summit are twofold: 1) to provide an update on new approaches to the genetic modification of large animal species for development of better animal models for biomedical research, biomanufacturing, and improved agricultural production; and 2) to provide a forum for discussions on funding mechanisms and regulations effecting development of genetically engineered large animals. Our first summit is being held in Bethesda, Maryland, to facilitate participation by representatives from various federal agencies who either fund or regulate the production of genetically engineered animals. We are grateful to the representatives from NIH, USDA and FDA who have agreed to participate in the meeting. We thank our sponsors who have helped make the summit possible and the people who have worked hard to make sure that the summit is a success. We trust that the summit will be informative, invigorating and intellectually stimulating. We also hope that this event will provide an opportunity for you to renew old friendships, as well as develop new contacts and collaborations.

Providing there is sufficient interest, our plan is for the LAGE Summit to become a biennial event. Consequently, we have already begun planning the second LAGE Summit, which we will host in Park City, Utah, during the summer or early fall of 2018. We hope that you will all be able to join us in Park City in 2018!

Please enjoy the summit and let us, or a member of the meeting staff, know if we can be of assistance in any way.

Sincerely,

Irina Polejaeva and Chris Davies
Co-Chairs, LAGE Summit 2016
Sunday, September 18

4:00 - 6:30 p.m. Registration, Congressional Ballroom

6:00 - 8:00 p.m. Opening Reception, Congressional Ballroom

Monday, September 19

6:30 - 8:00 a.m. Continental breakfast, Congressional Ballroom
7:00 a.m. - 5:00 p.m. Registration, Congressional Ballroom

8:00 - 8:15 a.m. Kenneth White, PhD, Utah State University, USA
Summit Welcome and Opening Remarks

8:15 - 9:00 a.m. Michael Roberts, PhD, University of Missouri, USA
Keynote Lecture - The Current Status of Transgenic Farm Animal Research

Session 1: Gene Editing Technology and Application
Session Chair – Bruce Whitelaw, PhD, Roslin Institute, United Kingdom

9:00 - 9:30 a.m. Dan Carlson, PhD, Recombinetics Inc, USA
Efficient Genome Editing in Livestock

9:30 - 10:00 a.m. Göetz Laible PhD, AgResearch, New Zealand
Technological Advances Create New Opportunities for the Targeted Improvement of Livestock

10:00 - 10:30 a.m. Bhanu Telugu, PhD, University of Maryland, USA
Engineering Genomes of Domestic Pigs for Agricultural Applications

10:30 - 10:45 a.m. Poster exhibit/beverage break, Senate Suites

Session 2: Modification of Disease Resistance and Susceptibility in Large Animals
Session Chair – Chris Davies, DVM, PhD, Utah State University, USA

10:45 - 11:15 a.m. Kevin Wells, PhD, University of Missouri, USA
Improved Resistance to Livestock Disease through Genetic Enhancement

11:15 - 11:45 a.m. Jorge Piedrahita, PhD, North Carolina State University, USA
Development and Characterization of Severe Combinare Immune Deficient Swine

11:45 a.m. - 12:15 p.m. Eddie Sullivan, PhD, SAb Biotherapeutics, USA
The Use of Genetically Engineered Large Animals to Rapidly Respond to Emerging Infectious Disease through the Production of Therapeutic Antibodies

12:15 - 1:15 p.m. Networking lunch – Congressional Ballroom
Session 3: Transgenic Large Animal Models
Session Chair - Carol Keefer, PhD, University of Maryland, USA

1:15 - 1:45 p.m. Irina Polejaeva, PhD, Utah State University and Ravi Ranjan, MD, PhD, University of Utah, USA
*Transgenic Goat Model of Atrial Fibrillation*

1:45 - 2:15 p.m. Randy Prather, PhD, University of Missouri, USA
*Development of Animals Models and National Swine Research and Resource Center*

Selected abstract presentations
2:15 - 2:30 p.m. Amy Desaulniers, University of Nebraska, USA
*Use of Genetically Engineered Swine to Elucidate Testicular Function in the Boar*

2:30-2:45 p.m. Seon-Ung Hwang, Chungbuk National University, Republic of Korea
*Production of Transgenic Pigs with pGFAP-CreERT2; EGFPloxP Inducible System*

2:45 - 3:45 p.m. Poster session/refreshment break, Senate Suites

Session 4: *Regulation of Genetically Engineered Large Animal Models and Medical Device Development Tools*
Moderator - Mark Walton, PhD, Yorktown Technologies, LP, USA

Presentations followed by a panel discussion.
3:45 - 4:15 p.m. Malini Wileman
4:15 - 4:45 p.m. Donna Lochner
4:45 - 5:15 p.m. Panelists include:
Malini Wileman, PhD, Center for Veterinary Medicine, FDA, USA
Donna Lochner, Center for Devices and Radiological Health, FDA, USA
Laura Epstein, J.D., Center for Veterinary Medicine, FDA, USA
Jill Ascher, DVM, MPH, Division of Veterinary Services, Center for Biologics Evaluation and Research, FDA, USA.

Tuesday, September 20

6:30 – 8:00 a.m. Continental breakfast, Congressional Ballroom
7:00 - 10:00 a.m. Registration, Congressional Ballroom

Session 5: Current and Future Large Animal Models in Biomedical Research
Session Chair - Zhongde Wang, PhD, Utah State University, USA

8:00 - 8:30 a.m. David Ayares, PhD, Revivicor Inc., USA
*Genetically Engineered Pigs for Xenotransplantation*

8:30 - 9:00 a.m. Michael Cutler, DO, PhD, Intermountain Heart Institute, USA
*Translational Cardiovascular Research Utilizing Large Animal Genetic Engineering*
9:00 - 9:30 a.m. Chris Rogers, PhD, Exemplar Genetics, USA
*TP53 and KRAS Targeted Pigs: A Platform for Models of Human Cancer*
Selected abstract presentations
9:30 - 9:45 a.m. Timothy Sheets, University of Maryland, USA
*Investigating the Conserved Role of Neurogenin 3 (NGN3) in Endocrine Pancreatic Development in the Porcine Model Using CRISPR/Cas9 System*
9:45 - 10:00 a.m. Min Yang, Utah State University, USA
*Introduction of Callipyge Mutation into the Goat Genome Using Transcription Activator-like Effector Nucleases (TALENs).*

10:00 - 10:30 a.m. Poster session/beverage break, Senate Suites

**Session 6: Funding Mechanisms to Support Genetically Engineered Livestock Research**
Moderator - Kenneth White, PhD, Utah State University, USA

Presentations followed by a panel discussion
10:30 – 10:50 a.m. Ravi Ravindranath
10:50 – 11:10 a.m. Adele Turzillo
11:10 – 11:30 a.m. Miguel Contreras
11:30 a.m. – 12:00 p.m. Panelists include:
Ravi Ravindranath, DVM, PhD, National Institutes of Health, Reproductive Sciences Branch, USA
Adele Turzillo, USDA, National Institute of Food and Agriculture, USA
Miguel Contreras, PhD, National Institutes of Health, Office of Research Infrastructure Programs, USA
Michael Roberts, PhD, Curator’s Professor of Biochemistry and Animal Sciences, University of Missouri, Member of the National Academy of Sciences, USA

12:00 - 1:00 p.m. Networking lunch, Congressional Ballroom

**Session 7: Current and Future Large Animal Models in Biomedical Research**
Session Chair - Jon Oatley, PhD, Washington State University, USA

1:00 - 1:30 p.m. Eckhard Wolf, University of Munich Gene Center, Germany
*Genetically Tailored Pig Models for Translational Diabetes Research*

1:30 - 2:00 p.m. Ann Harris, PhD, Feinberg School of Medicine, Northwestern University, USA
*Why Might New Large Animal Models of Cystic Fibrosis Be Useful for Generating New Therapies?*

2:00 - 2:30 p.m. Noelle Cockett, PhD, Executive Vice President and Provost, Utah State University, USA
*Future Perspectives and Closing Remarks*
Summit Welcome and Opening Remarks
Dr. Kenneth White, PhD, Utah State University, USA

Ken White is dean of the College of Agriculture and Applied Sciences at Utah State University, the vice president for USU Extension and director of the Utah Agriculture Experiment Station. Dr. White will open the summit and introducing the keynote speaker. Dr. White is known for his partnership in creating the first successful equine clones. He is also interested in research of early development and reproduction.

Keynote Lecture
Michael Roberts, PhD, University of Missouri

SI: The Current Status of Transgenic Farm Animal Research

R. Michael Roberts1, 2
1Division of Animal Sciences and, University of Missouri, Columbia, MO, USA; 2Bond Life Sciences Center, University of Missouri, Columbia, MO, USA

I will provide a brief and general account of transgenic research in farm animals, beginning with approaches that introduced genetic material directly into a pronucleus of the fertilized egg and concluding with gene editing by CRISPR/Cas9 technologies. Following this introduction, I’ll cover the fraught topic of pluripotent stem cells, including the perceived promise of such cells for transgenic research and the disappointing outcomes that have followed the initial enthusiasm. I’ll conclude this section with an account of why I believe such cells have continued relevance for both biomedical and agricultural purposes and present recent data from mine and Dr. Randall Prather’s laboratory on an improved means for in vitro maturation (IVM) of porcine oocytes. It is generally recognized that the attainment of development competency in in vitro-derived oocytes continues to be a challenge for assisted reproductive technologies (ART) in agriculturally important, large farm species, because IVM oocytes are less competent than their in vivo counterparts, particularly if they are derived from immature animals. I shall describe how three growth factors (FGF2, LIF, IGF-1), when added to an otherwise standardized IVM medium, are able to double the number of high-quality blastocysts that can be generated from a set number of oocytes derived from immature gilts. These oocytes provide close to normal size litters of healthy piglets following either somatic cell nuclear transfer or CRISPR/Cas9 gene editing in the zygote. These cytokines work synergistically in promoting oocyte IVM and induce hitherto unnoticed fluctuations of MAPK concentrations in cumulus cells (CCs) investing the oocytes, a phenomenon that appears to underpin the observed improvement in oocyte competence.

Michael Roberts is a Curators’ Professor of biochemistry and animal sciences at the University of Missouri. He is a member of the National Academy of Sciences and internationally recognized for the discoveries of interferon-tau and other pregnancy-associated proteins. He has published over 300 papers in refereed scientific journals and over 70 reviews and book chapters. Dr. Roberts also received the prestigious Milstein Prize for Research on interferons and the Wolf Prize in Agriculture.
Session 1 - Gene Editing Technology in Livestock and Applications

Session Chair - Bruce Whitelaw, PhD, Roslin Institute, United Kingdom

Bruce Whitelaw was awarded a Bachelor of Science degree in Medical Microbiology from the University of Edinburgh in 1982 and his PhD in 1987 from the University of Glasgow. Currently, Bruce is head of Division of Developmental Biology at The Roslin Institute and professor of Animal Biotechnology at the Royal (Dick) School of Veterinary Studies. Having pioneered the use of lentivirus vectors for transgene delivery, he is currently establishing robust methodology for genome editing in livestock.

Dan Carlson, PhD, Recombinetics Inc.

*S2: Efficient Genome Editing in Livestock*

Daniel F. Carlson¹, Adrienne L. Watson¹, Dennis A. Webster¹, Scott C. Fahrenkrug¹
¹Recombinitcics Inc., St. Paul, Minnesota, USA

Livestock harboring directed genetic compositions have tremendous potential for improvement of agricultural animals/products, regenerative medicine technologies and creation of large-animal models for preclinical assessment of therapeutics for safety and efficacy. A brief overview of editing platforms in livestock will be discussed followed by three examples where the technology was used to produce three “personalized” animal models of disease. Dilated cardiomyopathy (DCM) is a major cause of heart failure and a significant source of mortality and morbidity for children and adults. To create this model, we have leveraged the discovery of human mutations in RNA Binding Motif 20 (RBM20), an RNA-splicing protein. Pigs homozygous for the human mutant allele RMB20 R636S have severe and rapid onset of DCM leading to prototypical heart failure with reduced ejection fraction and fibrotic myocardium. Heterozygous animals suffer from a more latent form of disease consistent with heart failure in adult patients. The NF1 pig was developed by engineering a premature termination codon into minipigs that mimics a common disease allele in patients. We have observed a striking 100% penetrance of café au lait spots in these pigs, a phenotype that is seen in patients, but has never been demonstrated in any other animal model. Fibroblasts isolated from NF1 pigs also show hyperactive Ras activity, the underlying mechanism of human disease. Progress in characterization of other typical clinical presentations in NF1 patients will be reported. Complimentary to gene editing, transposon transgenesis is also a reliable tool for delivery of humanized mutant alleles. Transgenic Ossabaw expressing a gain-of-function chimp PCSK9 transgene have significantly increased cholesterol (5 to 15-fold, depending on diet) that leads to highly occlusive, mature atherosclerotic plaques characterized by high levels of inflammation, calcification and necrosis within the core of the coronary lesions.

Dan Carlson is a “farm kid” from southwestern Minnesota who interest in biotechnology when his family began planting genetically modified crops in the 90s. He attended the University of Minnesota where he earned a PhD in animal science with an emphasis in biotechnology and molecular genetics. The focus of his research is the refinement and application of methodology for genetic engineering in livestock. Through his 13 years in biotechnology research, Dan has led the development of transposon systems and gene-
Göetz Laible, PhD, AgResearch

S3: Technological Advances Create New Opportunities for the Targeted Improvement of Livestock

Göetz Laible¹, Brigid Brophy¹, David Wells¹, Sally Cole¹, Marion Wright¹, Stephanie Delaney¹, Ali Cullum¹, Aaron Malthus¹, Jingwei Wei¹, Stefan Wagner¹

¹ Animal Science, Reproduction, AgResearch Ruakura, Hamilton, New Zealand

Over millennia humans have modified the genetic makeup of livestock by breeding and selection strategies to better meet the need for food and other animal products. Although this strategy has been proven to be highly successful, improvements are generally limited to incremental steps and typically require the accumulation of small gains over long periods to realize genuine benefits. The development of transgenic livestock technology now provides a powerful alternative where it is possible to directly introduce genetic modifications with known impact and potentially achieve benefits within a single generation. While initially the technology was held back by technical limitations, these have been largely overcome by progressive improvements over the past 30 years. In particular, the development of site-specific nucleases facilitating the efficient and precise editing of livestock genomes makes it now possible to directly introduce and accumulate favourable alleles in the best animals. This next generation breeding approach was recently exemplified with the production of hornless dairy cattle.

Considering the importance of milk as a human food, the rational improvement of milk composition of dairy animals was one of the earliest goals with the development of transgenic livestock capabilities. The newly available precision tools create exciting new options for genetically engineering milk. The presentation will provide an overview about the humble beginnings and what might be possible now and in the near future to improve milk for human consumption with these new technologies.

Göetz Laible is a senior scientist at AgResearch, New Zealand’s largest Crown Research Institute. He holds a PhD in biochemistry from the Free University of Berlin, Germany and carried out his postdoctoral research at the Salk Institute for Biological Studies in San Diego. He leads a research program focused on the development and evaluation of genome modification technologies for future livestock applications.
The breeding of domestic animals has a longstanding and successful history, starting with domestication several thousand years ago. Modern animal breeding strategies predominantly based on population genetics, artificial insemination (AI) and embryo transfer (ET) technologies have led to significant increases in the performance of domestic animals, and are the basis for regular supply of high quality animal derived food at acceptable prices. However, the current strategy of marker-assisted selection and breeding of animals to introduce novel traits over multiple generations is too pedestrian in responding to unprecedented challenges such as changing climate, global pandemics, and feeding an anticipated 33% increase in global population in the next three decades. Here, we propose site-specific genome editing technologies as a basis for “directed” or “rational selection” of agricultural traits. These genome editing tools are expected to facilitate targeted modification of individual traits without affecting the overall genetic merit of the animal thereby ushering the animal biotechnology into the functional genomics era. The animal science community envisions these technologies as essential tools in addressing critical priorities for global food security and environmental sustainability, and strives to develop these technologies for maximum societal benefit. This work is supported by funding from NIH-NIFA Dual Purpose with Dual Benefit Grant # 2015-67015-22845.

Bhanu Telugu’s primary appointment is with the University of Maryland, where he is an assistant professor in the Department of Animal and Avian Sciences. He also holds a “visiting scientist” appointment with the USDA’s Agricultural Research Service. His laboratory employs genome-editing tools such as CRISPRs and TALENs for site-specific alterations of the pig genome for biomedical and agricultural applications.
Session 2: Modification of Disease Resistance and Susceptibility in Large Animals

Session Chair - Chris Davies, DVM, PhD, Utah State University, USA

Chris Davies is a research associate professor in the Department of Animal, Dairy and Veterinary Sciences at Utah State University, director of the university’s Center for Integrated BioSystems and associate director of the Utah Agricultural Experiment Station. His research interests include immune responses and gene expression at the maternal-fetal interface. Additionally he is interested in the identification and characterization of genes affecting immune function and disease resistance.

Kevin Wells, PhD, University of Missouri

S5: Improved Resistance to Livestock Disease through Genetic Enhancement

Kevin D. Wells

Division of Animal Sciences, University of Missouri, Columbia, MO, USA

It has been more than three decades since genetic engineering (GE) technologies were first adapted for use in livestock. The earliest attempts to apply GE to agricultural phenotypes were related to growth. In part, this focus was due to limitations in knowledge of gene function and the role of genes in physiological phenomena. However, GE efforts soon expanded to include disease resistance phenotypes. In the first decade of livestock GE, most of the real success was related to gleaned insight into the design of transgenes. Examples of health-related transgenes included MX1, specific immunoglobulins, and viral proteins. However, pronuclear microinjection proved to be inefficient and cost-prohibitive for many researchers, thus restricting the pace of GE. After the advent of animal cloning via somatic cell nuclear transfer, the costs of GE animal production was reduced. Among the first applications of the technology was again disease resistance, specifically, mastitis resistance. Recently, with the advent of designed nucleases (CRISPR technology in particular), not only was the cost of entry reduced, but the required skill level was also reduced. As a result, more groups (commercial and academic) have the capacity to modify livestock genomes. Since these new genome-editing technologies include the ability to make small changes that do not introduce transgenes, commercial interests have entered into the field with the hope of producing agricultural animals with economically viable phenotypes. This hope spurs from the assumption that small genome changes will be more acceptable to government regulators and/or the public. Although acceptance of GE in food animals remains unknown, it is clear that GE technologies have matured to the point that useful, viable animals can be produced. In addition, there are examples of disease resistance phenotypes that cannot currently be addressed by any other technology. The future of animal agriculture may depend entirely on the how regulators react to the very real benefits that GE livestock present. In addition, genetic engineers need to proceed carefully as to not select for resistant pathogens or create new pathogen reservoirs.
Kevin Wells received his PhD in genetics from North Carolina State University. He now serves as an assistant professor at the University of Missouri with research appointment in the College of Agriculture, Food and Natural Resources.

Jorge Piedrahita, PhD, North Carolina State University

**S6: Development and Characterization of Severe Combine Immune Deficient Swine**

Renan B. Sper & Jorge A. Piedrahita. Comparative Medicine Institute and College of Veterinary Medicine, North Carolina State University.

The goal of engrafting human cells into other species, in particular hematopoietic stem cells, has been pursued for six decades. This goal remains and there are multiple labs across the world that are using, and improving humanized mice to address a range of high impact biomedical questions. However there is a need for developing systems in species that more closely resemble humans in size, physiology and longevity. The pig offers an exceptional and perhaps the best system for this purpose. Pigs are widely available and the life span, and cell and organ physiology more closely approximates those of human. Pigs are readily bred, born in litters and amenable to genetic engineering. Additionally, recent advances in gene editing have allowed the development of complex multi-transgenic pigs at very high efficiencies. We will describe the development of a IL2RG/RAG1 deficient pig and its utilization for allogenic and xenogeneic engraftment.

Jorge Piedrahita is a professor in the Department of Molecular Biomedical Sciences in the College of Veterinary Medicine at North Carolina State University. He is also the director of the Comparative Medicine Institute, a university-wide center that incorporates over 160 faculty members from 23 different departments and five colleges.

Eddie Sullivan, PhD, SAB Biotherapeutics

**S7: The Use of Genetically Engineered Large Animals to Rapidly Respond to Emerging Infectious Disease through the Production of Therapeutic Antibodies**

Eddie J. Sullivan¹, Hua Wu¹, Jerry Pommer¹, Jin-An Jiao¹

¹SAB Biotherapeutics, Inc., Sioux Falls, SD, USA

SAB Biotherapeutics (SAB) has successfully developed a unique transchromosomic (Tc) bovine platform that can rapidly produce potent, fully human, immunoglobulins against a variety of disease targets including viruses and bacteria in significant quantities (up to 600g/month/animal of highly purified immunoglobulin). Several forms of passive immunotherapy to prevent and treat viral infection have and are being studied in animals. Convalescent plasma have been used in humans, but logistical and production limitations have prevented widespread use. Additionally, convalescent plasma and specific monoclonal antibodies to viruses are unlikely to have significant cross-protection efficacy against other strains. To overcome these limitations, we propose the use of a novel platform to rapidly produce and deliver high-titer human polyclonal immunoglobulin for treatment and post-exposure prophylaxis of viruses and other infectious and toxin-mediated diseases. SAB has
developed a Tc bovine in which the bovine immunoglobulin genes have been knocked out and a human artificial chromosome (HAC) containing the full germ line sequence of human immunoglobulin has been inserted. Each Tc bovine can produce 150 to 600 grams per month of purified immunoglobulin and the platform is rapidly scalable. Experimental high-titer immunoglobulins have been rapidly produced against tri-valent seasonal influenza (pH1N1, H3N2, type B), bi-valent hantavirus (Andes, Sin Nombre), tri-valent alphavirus (VEE, EEE, WEE), MERS-CoV, and anthrax, among others.

**Eddie Sullivan** is the president and CEO of SAb Biotherapeutics, a company that produced the world’s first large animal species that produces fully human polyclonal antibodies. Eddie has been a thought leader in this field and worked with various committees and discussion groups that have focused on animal biotechnology and has actively participated in discussions for establishing a regulatory framework for this technology.
Session 3: Transgenic Large Animal Models

Session Chair - Carol Keefer, PhD, University of Maryland, USA

Carol Keefer has done pioneering work in the development of in vitro production techniques in cattle, including in vitro fertilization, intracytoplasmic sperm injection, and nuclear transfer. She is noted for work that produced the first clone goats using in vitro transfected donor cells. Dr. Keefer is on the editorial board of Cloning & Stem Cells and is a past president, vice president and member of the board of governors of the International Embryo Transfer Society.

Irina Polejaeva, PhD, Utah State University
Ravi Ranjan, MD, PhD, University of Utah

S8: TGF-β1 Transgenic Goat Model of Atrial Fibrillation

Irina A. Polejaeva 1, Ravi Ranjan2, Christopher J. Davies 1, Kenneth L. White 1
1 Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA; 2 Division of Cardiology, University of Utah, Salt Lake City, UT, USA

Large animal models of progressive atrial fibrosis provide an attractive platform to study the relationship between structural and electrical remodeling in atrial fibrillation (AF). Here we established a new transgenic goat model of AF with cardiac specific overexpression of TGF-β1 and investigated the changes in the cardiac structure and function leading to AF. Transgenic goats with cardiac specific overexpression of constitutively active TGF-β1 were generated by somatic cell nuclear transfer. We examined myocardial tissue, ECGs, echocardiographic data, and AF susceptibility in transgenic and wildtype control goats. Transgenic goats exhibited a significant increase in fibrosis and myocyte diameter in their atria compared to controls, but not in their ventricles. P-wave duration was significantly greater in transgenic animals starting at 12-months of age, but no significant chamber enlargement was detected, suggesting conduction slowing in the atria. Furthermore, this transgenic goat model exhibited a significant increase in AF vulnerability. Six of eight transgenic goats (75%) were susceptible to AF induction and exhibited sustained AF (>2 minutes), whereas, none of six controls displayed sustained AF (P<0.01). Length of induced AF episodes was also significantly greater in the transgenic group compared to controls (687±212.02 vs. 2.50±0.88 seconds, P<0.0001).

Marked atrial fibrosis, P-wave prolongation and a significant increase in AF susceptibility (following a brief electrical induction) are the primary hallmarks of this TGF-β1 transgenic goat model. In humans, the prevalence of AF increases with age due to atrial substrate remodeling developing slowly over many decades of life. These transgenic goats for the first time provide a large animal model where progressive accumulation of fibrosis creates the basis for developing AF. Serial electrophysiological studies done over time with fibrosis progression in this model can lead to further mechanistic understanding of AF. We suggest that these studies will open new clinically relevant mechanism-based targets for the prevention and treatment of AF.

Irina Polejaeva is an associate professor in the Department of Animal, Dairy and Veterinary Sciences at Utah State University and a member of Utah Multidisciplinary Arrhythmia Consortium. Dr. Polejaeva is also a member of the Veterinary Diagnostics and
Infectious Disease USTAR program. She served for 8 years as chief scientific officer at ViaGen Inc., led the Cell Biology Group and was project manager for the Porcine Nuclear Transfer Program at PPL Therapeutics Inc. Her work led to generation of the world’s first cloned pigs by somatic cell nuclear transfer (SCNT) and the birth of the world’s first 1, 3-galactosyltransferase deficient pigs. Her primary emphasis is development of GE large animal models for biomedical research.

Ravi Ranjan, M.D., Ph.D., is a cardiologist specializing in cardiac electrophysiology. His clinical interests include ablation of complex cardiac arrhythmias like atrial fibrillation, ventricular tachycardias and supra-ventricular tachycardias. He also has keen interest in cardiac device based therapy like cardiac resynchronization therapy, ICDs and pacemakers. He has been a faculty member at the University of Utah since 2010. He received his medical degree from Harvard Medical School, completed an internal medicine residency at Massachusetts General Hospital, Harvard Medical School, a cardiology fellowship at Johns Hopkins Hospital and a cardiac electrophysiology fellowship at Johns Hopkins Hospital. Dr. Ranjan has built a large clinical cardiology and electrophysiology practice in Utah.

Randy Prather, PhD, University of Missouri

**S9: Development of Animal Models and National Swine Research and Resource Center**

Randall S. Prather, Kevin D. Wells
Division of Animal Sciences, National Swine Resource and Research Center, University of Missouri, Columbia, MO, USA

There is an urgent need from human medicine, basic biology and production agriculture for genetically engineered (GE) swine. Human medicine demands models that faithfully replicate the human condition to develop treatments, and to practice techniques to cure human ailments. In some cases, swine are by nature appropriate, e.g. cardiovascular disease, etc. However, in other cases GE makes swine the species of choice for modeling humans to characterize development and progression of disease, and to develop treatments and therapies, e.g. cystic fibrosis, diseases of retinal degeneration, etc. A human sized model is necessary for developing devices, stem cell therapy strategies, and scaling treatments. Swine are the species of preference for providing organs for xenotransplantation to humans. The National Institutes of Health (NIH) has considered swine important enough to their mission of meeting the critical need of models that it established the National Swine Resource and Research Center (NSRRC). The NSRRC imports models created by others, curates models (as both live animals and frozen genetics), creates models as requested by the NIH community, and distributes cells tissues and animals to other not-for-profits. Applications of GE swine to areas other than medicine are equally justifiable. These range from understanding basic animal physiology, improving productivity, decreasing inputs, and changing the carcass composition, to making animals resistant to disease. GE of domestic pigs can help to address threats to our food security, and improve both sustainability and animal welfare. One recent example is that of using gene editing to make pigs resistant to porcine reproductive and respiratory syndrome virus (PRRSV). PRRSV costs $6M/day in North America and Europe. The potential economic, sustainability, and animal welfare benefits clearly justify GE swine for applications beyond resistance to PRRSV. We propose that the United States Department of Agriculture
establish a National Agricultural Swine Resource and Research Facility (NASRRF) that would focus on GE of swine. The NASRRF would respond to agricultural researchers across the country to provide not only models to better understand basic physiology, but also to make animals that would address the important issues facing the swine industry. Clearly, the technology is now advanced to the point where investments need to be made in GE of swine for both production agriculture and human medicine.

Randy Prather is currently a Curators’ Professor with the title of Distinguished Professor of Reproductive Biotechnology in the Division of Animal Science at the University of Missouri. He is also the director of the NIH-funded National Swine Resource and Research Center. His lab has made over 1000 cloned pigs at MU representing over 45 different genetic modifications for agriculture and medicine.

Amy Desaulniers, PhD Student, University of Nebraska

S10: Use of Genetically Engineered Swine to Elucidate Testicular Function in the Boar

Amy T. Desaulniers, Rebecca A. Cederberg, Clay A. Lents, and Brett R. White

The second mammalian GnRH isoform (GnRH-II) and its specific receptor (GnRHR-II) are highly expressed in the testis. Coding errors prevent their production in many species, but both genes are functional in swine. We demonstrated that GnRHR-II localizes to porcine Leydig cells and GnRH-II stimulates testosterone production in vivo, despite negligible luteinizing hormone (LH) secretion. These data indicate that GnRH-II directly effects testicular steroidogenesis. To explore this hypothesis, we produced a GnRHR-II knockdown (KD) swine line. Upon evaluation during pubertal development, testosterone concentrations were reduced in transgenic compared to littermate control males. However, LH was unaffected, suggesting that GnRHR-II KD impairs steroidogenesis directly at the testis rather than inhibiting gonadotropin secretion. Based on these results, our next objective was to compare diurnal secretory patterns of testosterone in mature transgenic (n = 5) and littermate control (n = 5) males. Boars were fit with indwelling jugular cannulae and blood was collected every 15 min for 8 h. Serum testosterone concentrations were quantified via radioimmunoassay. Next, males were euthanized; testis weight was recorded and testicular tissue collected to confirm GnRHR-II KD in transgenics via digital droplet PCR (normalized to β-actin). Results revealed that testosterone was unaffected by time or line x time interaction (P > 0.05), but a line effect was detected (P = 0.04). Differences were dramatic; testosterone levels were reduced by 82% in GnRHR-II KD (0.75 ± 0.05 ng/ml) compared to control (4.09 ± 0.29 ng/ml) males. Despite divergent testosterone concentrations, testis weights were similar between lines (P > 0.05), suggesting diminished Leydig cell function versus hypotrophy/hypoplasia. Since testicular GnRHR-II mRNA levels were reduced by 69% in transgenics (P < 0.001), these data demonstrate that GnRH-II and its receptor play a critical role in testosterone biosynthesis within porcine Leydig cells. Given that testosterone and its metabolites dictate male reproductive success, GnRH-II and its receptor represent unique targets to improve boar fertility. Notably, these swine represent the first genetically engineered animal model to examine the role of GnRH-II and
its receptor in mammals. Supported by NIFA Hatch (NEB-26-199; BRW) and AFRI (2011-67015; CAL) funds. USDA is an equal opportunity provider and employer.

Seon-Ung Hwang, Chungbuk National University, Korea

*11: Production of Transgenic Pigs with pGFAP-CreERT2; EGFPLoxP Inducible System*

Seon-Ung Hwang1,2, Junchul David Yoon1,2, Kiyoung Eun3, Hyunggee Kim3,* and Sang-Hwan Hyun1,2*

1Laboratory of Veterinary Embryology and Biotechnology, Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea; 2Institute of Stem Cell & Regenerative Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea; 3Department of Biotechnology, School of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea.

Transgenic (TG) pigs are currently believed to be an important model for biomedical research, including for disease models, pharmaceutical toxicity testing and regenerative medicine. In the present studies, we constructed two vector systems using pGFAP promoter; one retains pGFAP promoter-CreERT2 transgene and the other has enhanced green fluorescent protein (EGFP) gene flanked by LoxP sites which can be eliminated through the CreERT2-mediated recombination. We also established donor transgenic pig fibroblasts with pGFAP-CreERT2+LCMV-EGFPLoxP for somatic cell nuclear transfer (SCNT). Then, to produce TG pigs with pGFAP-CreERT2; LCMV-EGFPLoxP transgene constructs, embryo transfer was performed three times just before ovulation state of the surrogate mothers. One of them was pregnant, and delivered five TG pigs at 115 days after pregnancy. Polymerase chain reaction (PCR) analysis with genomic DNA prepared from skin tissues of TG pigs revealed that all five TG pigs had transgenes. In addition, EGFP expression in all organs tested was confirmed by autopsy and PCR. The real-time PCR analysis showed that pGFAP promoter-driven CreERT2 mRNA was highly expressed in the cerebrum. Three months after birth, TG pig was orally administrated with 15 mg/kg of 4-hydroxytamoxifen (TM) during 5 days, and then euthanized after 7 days. Semi-nested PCR analysis with genomic DNA revealed that the CreERT2–mediated recombination was induced in the cerebrum and cerebellum, but not skin. Taken altogether, we generated TG pig with TM-inducible pGFAP-CreERT2; EGFPLoxP recombination system by SCNT. The current study is the first report of the astrocyte-specific pGFAP-CreERT2 inducible system in TG pigs. Therefore, we suggest that this technology will be a useful tool for studying physiology of astrocytes and generating TG pig model of CNS diseases such as Huntington’s disease, Alzheimer’s disease and brain tumor. Keywords: Porcine, SCNT, CreERT2 system, GFAP This work was supported, in part, by a grant from the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011288, PJ011077)" Rural Development Administration, and the "National Research Foundation of Korea Grant funded by the Korean Government (NRF-2013R1A2A2A04008751)", and the "Ministry of Trade, Industry & Energy(MOTIE), Korea Institute for Advancement of Technology(KIAT) through the Leading Industry Development for Economic Region (Project No. R0004357)", Republic of Korea.
Session 4: Regulation of Genetically Engineered Large Animal Models and Medical Device Development Tools, Presentations and Panel Discussion

Moderator - Mark Walton, PhD, Yorktown Technologies, LP, USA

Mark Walton is a veteran of the plant and animal biotechnology industries. He has worked as a research scientist, entrepreneur and corporate executive. In 2016 he joined Yorktown Technologies, LP, the developer of GloFish Fluorescent Fish, to lead a new division, Ornamental Bio. Previously he was the chief marketing officer for Recombinetics. Dr. Walton played a key role in obtaining the Cloning Risk Assessment and the Guidance on Regulation of Genetically Engineered Animals from the U.S. Food and Drug Administration.

Malini Wileman, PhD, Biologist, Center for Veterinary Medicine, FDA, USA

Dr. Wileman (née Mansharamani) is a highly skilled regulatory reviewer with technical expertise in cell and molecular biology, biochemistry, and biotechnology. She draws on her 9 years of combined regulatory experience in human and animal biologics development and manufacture as the lead scientist at the Center for Veterinary Medicine at the FDA, for several technical and policy-making issues, particularly emerging technologies, biopharm animal production, and inter-center collaboration and review. Dr. Wileman obtained her PhD in Biomedical Sciences from the Texas Tech University Health Sciences Center. After completion of her post-doctoral research at the Johns Hopkins School of Medicine in Baltimore, MD, Dr. Wileman worked in biologics manufacturing and process validation at BioReliance, and joined the FDA in 2009.

Donna Lochner, Senior Scientific Advisor, Center for Devices and Radiological Health, FDA, USA

Donna Lochner is associate director of the Office of Science and Engineering Laboratories at the FDA where she manages regulatory science initiatives. Prior to her current position, she was deputy director of the FDA’s Division of Cardiovascular Devices and chief of FDA’s Intraocular and Corneal Implants Branch. Before joining the FDA, Donna worked for Cordis Corporation. She earned a degree in chemical engineering from the Pennsylvania State University.

Laura Epstein, Senior Analyst, Center for Veterinary Medicine, FDA, USA

As a senior policy analyst for the U.S. Food and Drug Administration’s Center for Veterinary Medicine (CVM), Laura Epstein analyzes proposed policies and evaluates the effects of existing ones in order to help the FDA make informed decisions and achieve the center’s mission to protect human and animal health. The CVM approves animal drugs and monitors their safety and efficacy for companion and food-producing animals.
Jill Ascher, DVM, MPH, Deputy Director, Division of Veterinary Services, Center for Biologics Evaluation and Research, FDA, USA

As deputy director of the Division of Veterinary Sciences at the FDA, Dr. Ascher participates in planning, managing, organizing and directing activities of the division to safeguard animal welfare by providing exceptional veterinary care throughout the animal program. She is committed to protecting and improving public health, and ensuring the safety and effectiveness of new drugs through support of regulatory sciences research.
Session 5: Current and Future Large Animal Models in Biomedical Research

Session Chair - Zhongde Wang, PhD, Utah State University, USA

Dr. Zhongde Wang is an associate professor in the Department of Animal, Dairy and Veterinary Sciences at Utah State University. In addition to his academic appointment, Dr. Wang is also the president, chief scientific officer and co-founder of Auratus Bio, a biotech company specialized in animal genetic engineering. Dr. Wang’s research team employs modern genome engineering tools (such as CRISPR/Cas9, TALEN and piggyBac transposons) and assisted reproduction technologies (such as somatic cell nuclear transfer or cloning) to create genetically engineered animal models of human diseases. The Wang laboratory is the first to succeed in developing gene-targeting tools in the golden Syrian hamster. Dr. Wang received his Ph.D. degree in molecular and cellular biology from University of Massachusetts at Amherst in 2000 and did his postdoctoral trainings at Massachusetts Institute of Technology.

David Ayares, PhD, Revivicor Inc., USA

S12: Genetically Engineered Pigs for Xenotransplantation

David Ayares1, Todd Vaught1, Suyapa Ball1, Jeff Monahan1, Kasinath Kuravi1, Ben Morrill1, Amy Dandro1, Anneke Walters1, Steven Butler1, Lori Sorrells1, Kent Adams1, Maria Kokkinaki1, Jagdeece Ramsoondar1, John Bianchi1, and Carol Phelps1
1Revivicor Inc, Blacksburg, Virginia, USA (a subsidiary of United Therapeutics)

Genetic modification of the genome of pigs offers the opportunity to provide an unlimited supply of human-compatible xenograft donor organs, cells, and tissues for transplantation. Editing nucleases such as CRISPR/Cas9, combined with homology-driven recombination make gene knockout or knockin seem routine. Knockout of the α1,3-galactosyl transferase gene (GTKO) resulted in the elimination of hyperacute rejection (HAR), and prolonged survival of xenografts in non-human primate studies. Employing a combination of cloning and breeding, next-generation GTKO pigs were produced with constitutive high-level expression of the complement regulatory genes, hCD46 and hDAF, that demonstrated strong inhibition of complement mediated cell lysis, while in vivo studies in non-human primates with islets and whole organs showed prolonged survival and function (over 6mo. for both islets and hearts) in the absence of antibody-mediated rejection. In order to overcome molecular incompatibilities in clotting factors between donor and recipients, human transgenes for inhibition of coagulation and thrombosis, including TFPI, thrombomodulin (TBM), EPCR and CD39, under endothelial cell-specific promoter systems, were added alone or in combination to produce donor pigs. Hearts from GTKO/hCD46/TBM transgenic pigs transplanted into baboons have maintained function for 900+ days, (with two others exceeding 1 year), demonstrating significant efficacy of these pig organ xenografts. In addition, towards prevention of cell mediated rejection with reduced immuno-suppression, source pigs were produced which overexpress a dominant-negative inhibitor of SLA class II (CIITA-DN), as well as genes to provide cytoprotection (HO-1 and A20), and immunomodulation (CD47). Multi-cistronic “2A” vectors have facilitated the production of transgenic pigs with more than six genetic modifications,
where the added genes are not only co-expressed, but co-integrated in the genome, thus allowing propagation of new lines with reduced transgene segregation. Tissues, cells, and organs from these multi-transgenic pigs have the potential to address virtually all levels of xenograft incompatibility, and should help drive outcomes that justify initiation of human clinical trials.

David Ayares is executive vice president and chief scientific officer for Revivicor Inc. and leader of the team that cloned the world’s first pigs. Revivicor is a regenerative medicine company focused on a diverse pipeline of genetically engineered pigs as an alternative source of organs and tissues for human therapeutic use. Dr. Ayares was previously vice president of research and COO for PPL Therapeutics, and he worked for seven years in the pharmaceutical industry at Abbott Laboratories and Baxter Healthcare, developing transgenic mouse models for pharmaceuticals testing.

Michael Cutler, DO, PhD, Intermountain Heart Institute

**S13: Translational Cardiovascular Research Utilizing Large Animal Genetic Engineering**

Michael J Cutler, DO, PhD¹

¹Intermountain Medical Center Heart Institute, Intermountain Medical Center, Murray, Utah, USA

Cardiovascular disease is the #1 cause of death worldwide and is a major public health problem. Understanding the underlying mechanisms of cardiovascular disease is paramount to developing new and improved therapies to reduce the incidence of cardiovascular disease-related deaths. Significant advances have been made in our understanding of the cellular and molecular mechanisms of many aspects of cardiovascular disease using genetically altered small animal models. However, there are important limitations to translating these research findings to human medicine. This presentation will discuss the limitations of current small animal models and the potential benefits of large animal genetic engineering in advancing our understanding of the cellular and molecular mechanisms of cardiovascular disease. In particular, there will be special emphasis on the role of large animal genetic engineering in advancing these mechanistic insights into clinically meaningful therapies in humans.

Michael Cutler is a heart rhythm specialist at the Intermountain Heart Institute and was previously an assistant professor of medicine at Case Western Reserve University. His research interests include understanding the cellular and molecular mechanisms of cardiac arrhythmia and gene therapy for cardiac arrhythmias. Dr. Cutler has received recognition from the American Physiological Society, American Heart Association and the Heart Rhythm Society for his work.
We recently developed Yucatan miniature pigs with targeted TP53 mutations. Initial characterization reveals that 100% of TP53R167H/R167H mutant pigs develop cancer. These homozygous TP53 mutant pigs express two mutant alleles (R167H) of the p53 tumor suppressor in all tissues and are an outstanding model of osteosarcoma and B-cell lymphoma; however, the animals die from those tumors before other cancers can develop. By comparison, heterozygous TP53R167H/+ pigs are tumor-free after more than 2.5 years of age, though they are likely ‘primed’ for tumor development if other cancer genes are concomitantly altered. Our objective is to generate and characterize porcine cancer models in which tumorigenesis can be conditionally induced in specific tissues. We are particularly interested in lung and pancreatic cancers since they are among the deadliest and most difficult to diagnose malignancies in humans. A majority of human lung and pancreatic tumors are defined by the combined activation of oncogenic KRAS and inactivating mutations of TP53 (typically on just one allele). Mouse models that replicate those genetic events by conditionally expressing TP53 and KRAS mutations in the lung or pancreas effectively develop cancers in those tissues. While informative, such small animal tumor models possess significant limitations and challenges for translational oncology. Large animals, such as pigs, share greater similarities with humans in anatomy, physiology, genetics, lifespan, and importantly, size. Therefore, we created dual targeted KRASG12D/+/TP53R167H/+ pigs which express one mutant allele of the TP53 gene as well as Cre-inducible mutant KRAS. We hypothesize that these conditional KRAS/TP53 mutant pigs will produce site-specific cancers, as seen in comparable mouse models, providing much needed large animal tumor models for translational imaging, biomarker discovery, and pre-clinical testing of novel anticancer therapies. Studies are currently underway to establish the utility of the conditional KRASG12D/+/TP53R167H/+ pig model by defining optimal conditions for mutant KRAS induction and tumor development in the lung and pancreas.

Chris Rogers is the chief scientific officer and co-founder of Exemplar Genetics, a company focused on the development and study of improved models of human disease. Dr. Rogers received his PhD from Vanderbilt University and completed post-doctoral training at the University of Iowa. While at Iowa, Dr. Rogers and colleagues created the first knockout and knockin models of a human disease in a large animal species - cystic fibrosis in pigs. At Exemplar Genetics, Dr. Rogers has directed the development and characterization of additional disease models including cancer, neurodegenerative disorders and cardiovascular diseases.
The domestic pig is important as an agricultural and biomedical model species. The pig can serve as a complement to current mouse models, and in many cases, replace the mouse completely as a model for human disease. A failure to translate data generated from the mouse into human clinical trials limits the use of this model moving forward. The domestic pig is currently used in the medical device industry and by drug companies, due to its similar organ and vessel size to humans and the ability to model Adsorption Distribution, Metabolism and Excretion (ADME) studies. In the age of genome engineering and targeted gene therapy, the pig, can provide a longer life expectancy for disease progression, long term phenotypes and treatment end points. Using an example of ongoing disease modeling, the CFTR pig model not only surpasses the mouse models in comparative primary lung phenotype, but also recapitulates secondary phenotypes, specifically pancreatic agenesis leading to diabetes. In this study, we investigated whether the role of NGN3 is conserved across species. NGN3 is a member of the basic helix-loop-helix transcription factor family. In the mouse, Ngn 3 is both necessary and sufficient for the formation of endocrine pancreas during development. NGN3 is also believed to function in human endocrine pancreas development. In this study, we identified and amplified NGN3 from porcine BAC libraries. Once assembled, we designed guides to specifically target genomic sequence of NGN3 in the domestic pig for generating a knock-out. We injected a pre-complexed CRISPR/Cas9 protein and a NGN3-targeting sgRNA into porcine in vivo derived embryos; which were then transferred into synchronized surrogate animals. At day 63 of pregnancy, 9 fetuses were harvested for genotypic and phenotypic analysis. Out of 9 piglets, 5 of them had biallelic mutations across the target site. RT-PCR data show loss of downstream genes in 3 of the biallelic mutants. Currently, we are confirming these data with IHC. Once the role of NGN3 is confirmed, we will investigate its role in transdifferentiation, converting exocrine cells to an endocrine lineage, and evaluate their efficacy in vitro and in vivo.
The callipyge (CLPG) mutation arose in sheep in the 1980s with a phenotype of muscle hypertrophy and a novel inheritance mechanism of polar overdominance. The mutation is a single base, A to G transition, located within DLK1-DIO3 imprinted gene cluster. The regulatory mutation increases expression of several genes within the cluster. The aim of this study was to transfer the CLPG mutation into goats using TALENs and somatic cell nuclear transfer (SCNT). The desired adenine to guanine base change was introduced by co-transfection of TALENs and a single-stranded oligonucleotide was utilized as a template for homology directed repair. This strategy enabled production of homozygous and heterozygous cell clones for SCNT. CLPG cells were transferred into enucleated oocytes. Fused embryos were then activated for 5 min in 5 µM ionomycin followed by 4 h in 2mM DMAP with 5 µg/ml cycloheximide, cultured overnight and transferred into the oviducts of synchronized recipients. In total, 348 one-cell stage embryos were transferred to 21 recipient goats. Three pregnancies went to term and four male offspring that carried homozygous CLPG alleles were produced. These goats would not be expected to exhibit muscle hypertrophy but may have altered gene expression. Biopsies of the longissimus dorsi were used to assess gene expression in the DLK1-DIO3 cluster (4 clones and 6 age-matched controls). Gene expression was assayed for five loci (DLK1, CLPG1 and three maternal expressed long ncRNA) using RT-qPCR. There were no significant differences in gene expression between clones and controls. However, two clones had greater than 2-fold increase in the MEG3 levels and the other two clones had greater than 2-fold increased levels of DLK1 and much larger increases in CLPG1. The high variability in gene expression among the clones is likely due to incomplete reprogramming often observed following SCNT. These results demonstrate that TALENs and SCNT technologies can be efficiently used to introduce point mutations in goats. The muscle hypertrophy phenotype and regulatory effects of the goat CLPG allele will be tested by paternal transmission of the nascent allele to F1 progeny.
Session 6: Funding Mechanisms to Support Genetically Engineered Livestock Research, Presentations and Panel Discussion
Moderator - Ken White, PhD, Utah State University, USA

Ravi Ravindranath, DVM, PhD, NICHD, National Institutes of Health, USA

Ravi Ravindranath is director of the Preimplantation Genetics and Development Program in the National Institutes of Health’s Reproductive Sciences Branch. He is responsible for grants related to oocytes, sperm, fertilization, preimplantation embryo development, embryonic stem cells, and germ cell development. His portfolio also includes grants related to cloning and reprogramming, preimplantation genetic diagnosis and Assisted Reproductive Technologies (ART). Following his doctorate in veterinary medicine and a M.S. in microbiology, Ravi earned a PhD in biochemistry from the Indian Institute of Science. He completed postdoctoral training at the University of Pittsburgh and the Clinical Research Institute of Montreal, Canada, before joining the Department of Anatomy and Cell Biology faculty at the Georgetown University Medical Center, Washington, D.C. He was an adjunct scientist in the Laboratory of Clinical and Developmental Genomics at the National Institute of Child Health and Human Development. His research background is in reproductive biology and endocrinology of rodents and nonhuman primates.

Adele Turzillo, PhD, Director, Division of Animal Systems, USDA National Institute of Food and Agriculture, USA

Adele provides leadership in the development, evaluation and management of a broad range of programs in research, extension and education. She is a primary point of contact for issues related to livestock production and serves as liaison to academia, the states, and several multistate projects. Adele also interacts with other federal agencies and serves as a scientific advisor on matters related to animal agriculture.

Miguel Contreras, PhD, Program Officer, Division of Comparative Medicine, Office of Research Infrastructure Programs, National Institutes of Health, USA

Miguel Contreras is a program officer with the Division of Comparative Medicine, Office of Research Infrastructure Programs, located within the Division of Program Coordination, Planning, and Strategic Initiatives, National Institutes of Health Office of the Director. He manages a research resource grant portfolio that includes aquatic and mammalian models and is program coordinator for the SBIR/STTR Small Business program. He is a biochemist at the Universidad de Concepcion, Concepcion, Chile. He received his PhD in molecular and cellular biology and pathobiology from the Medical University of South Carolina (MUSC), in Charleston. He was a Fogarty International Fellow at the Laboratory of Neurosciences, National Institute on Aging, NIH, in Bethesda, MD. Prior to joining ORIP, he was an assistant professor at the Charles Darby Children’s Research Institute and the Department of Pediatrics, College of Medicine, of the MUSC. His research focused on the mechanism of degeneration of the nervous system’s white matter in inherited metabolic disorders that affect peroxisomes and lysosomes (X-linked Adrenoleukodystrophy, Globoid cell leukodystrophy).
Session 7: Current and Future Large Animal Models in Biomedical Research

Session Chair - Jon Oatley, PhD, Washington State University, USA

Dr. Oatley’s studies the regulation of germline cell fate decisions in mammalian testis. His current research focus is on investigating the role of basic helix-loop-helix (bHLH) proteins in controlling spermatogonial stem cell fate decisions, the influence of non-coding small RNAs on establishment of the spermatogonial stem cell pool, and identifying growth factors produced by testis somatic support cell populations that contribute to the niche microenvironment. He directs Washington State University’s Center for Reproductive Biology.

Eckhard Wolf, PhD, University of Munich Gene Center

S17: Genetically Tailored Pig Models for Translational Diabetes Research

Eckhard Wolf1,2, Andreas Blutke3, Rüdiger Wanke3, Simone Renner1,2

1 Gene Center and Center for Innovative Medical Models (CiMM), LMU Munich, Munich, Germany
2 German Center for Diabetes Research (DZD), Neuherberg, Germany
3 Institute of Veterinary Pathology, Center for Clinical Veterinary Medicine, LMU Munich, Munich, Germany

Diabetes mellitus (DM) has emerged into a steadily increasing health problem and the predicted future dimension of the global DM epidemic is alarming. Thus concerted research efforts are imperative to gain insight into disease mechanisms and to develop preventive and therapeutic strategies. Diabetic rodent models have limitations for translational research. Genetically engineered pig models may help to bridge the gap between basic research and clinical studies in (pre)diabetic patients (reviewed in [1]).

The incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP1) are secreted by enteroendocrine cells upon nutrient ingestion and stimulate insulin secretion. In type 2 diabetic patients the insulinotropic action of GIP is markedly reduced. To mimic this disturbance in a large animal model, we generated transgenic pigs expressing a dominant-negative GIP receptor (GIPRdn) under the control of rat Ins2 promoter sequences in the pancreatic islets (reviewed in [2]). GIPRdn transgenic pigs exhibit an impaired incretin effect due to a blunted insulinotropic action of GIP, a progressive deterioration of glucose control due to delayed and – at later stages – quantitatively reduced insulin secretion, and an impairment of physiological age-related expansion of beta-cell volume [3]. GIPRdn transgenic pigs thus provide a unique opportunity to screen for biomarker candidates during the pre-diabetic period [4] and to test therapeutic strategies targeting the GLP1 receptor [5].

Missense mutations in the INS gene have been identified as common cause of insulin-deficient, permanent neonatal diabetes mellitus in humans, also referred to as mutant INS gene-induced diabetes of youth (MIDY). We produced a transgenic MIDY pig line expressing INSC94Y under the control of the porcine INS promoter. MIDY pigs show early-onset clinical diabetes mellitus, markedly reduced body weight gain and beta-cell volume associated with a marked reduction of insulin secretory granules and severe dilation.
of the endoplasmic reticulum in the beta-cells [6]. MIDY pigs can be used for insulin treatment studies or for testing the efficacy of gene or cell therapies as well as islet transplantation. Secondary lesions of diabetes mellitus are another interesting area of research. We thus established the Munich MIDY pig biobank (highlighted in [7]) as a unique resource for studying systemic consequences of chronic hyperglycemia.


Funding: German Center for Diabetes Research (DZD); German Research Council (Transregional Collaborative Research Center 127).

**Eckhard Wolf** is director of the Laboratory for Functional Genome Analysis. He is the director of the Center for Innovative Medical Models, a member of the German National Academy of Sciences and corresponding member of the Austrian Academy of Sciences. Dr. Wolf is a leading specialist in the development of tailored animal models for translational biomedical research.

**Ann Harris**, PhD, Feinberg School of Medicine, Northwestern University

**S18: Why Might New Large Animal Models of Cystic Fibrosis be Useful for Generating New Therapies?**

**Ann Harris**, PhD, Dept. Pediatrics and Lurie Children’s Research Center, Northwestern University, Chicago, IL. USA

There are now many animal models of the common, inherited disorder cystic fibrosis (CF). These include multiple mouse strains, with various human pathogenic mutations on different genetic backgrounds; a rat; ferrets; rabbits and several mutant pig lines. Each of these models recapitulates some aspects of the human disease, but none accurately reproduces the complete human phenotype. Severity of disease in CF pigs, the most similar model to humans, presents additional challenges. These limitations are particularly relevant when considering the testing of new therapeutic strategies. Here we consider what additional models could advance understanding of the CF phenotype and how they might contribute to attempts to find a cure for CF.

**Ann Harris** is a professor in pediatrics and human molecular genetics at the Feinberg School of Medicine. Her primary research interest is elucidation of the regulatory mechanisms for the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which when mutated causes the devastating inherited disease cystic fibrosis.
Opportunities for genetic editing in livestock exist in the areas of large animal biomedical models and improvement of livestock production. While the generation of genetic edited animals is fairly straightforward, both areas are impeded by onerous regulatory processes that govern genetically modified animals. These regulations are primarily focused on the perceived dangers of consuming end products such as milk or meat of a genetically modified animal. Although large animals used as biomedical models are not intended for human consumption, the current regulations require containment to prevent the animal from moving into the food chain. In contrast, genetically edited production animals are intended for human consumption but pose no more risk to the consumer than any other production animals. In some cases, because of the genetic edit, animals will require less antibiotics and/or have reduced welfare issues, which are desirable outcomes for consumers.

Given the innocuous methods that now exist for genetic editing, the focus of regulation should move from the process of generating the animal to a review of the resulting animal. Refocusing regulation requirements will substantially reduce the cost of genetically edited animals. However, an additional cost is added to the large animal biomedical model, which is the need for the model to be validated using a collection of clinical phenotype measurements. It is unlikely that researchers will use a large animal model without that validation.

Noelle Cockett currently serves as Utah State University’s executive vice president and provost, and is a professor in the Department of Animal, Dairy and Veterinary Sciences. She has built a career in sheep genetics research, specializing in molecular genetics and identifying genetic markers. Dr. Cockett is known for being part of the research team that successfully sequenced the ovine genome.
**P1: A Simple and Efficient Vitrification Procedure for Cryopreserving Mouse Embryos and Oocytes Using Plastic Semen Straws**

Mo Guan, Martin Fray  
Medical Research Council, Mary Lyon Centre, Harwell Campus, Oxfordshire, OX11 0RD, UK

Vitrification is a freezing method in which cells and media are solidified without ice crystal formation. Consequently, injuries related to ice formation are less likely to occur. To achieve success in vitrification, high cooling rates and high concentrations of solutes are commonly believed to be necessary. However, to reduce the toxicity of the vitrification solutions and to achieve a high cooling rate, a minimum time in the vitrification solution and a minimum volume of vitrification solution are thought to be essential. However, these requirements make embryo/oocyte handling difficult during the vitrification, and subsequent warming procedures. Compared with other vitrification methods, the DAP213 vitrification method is simple and efficient, and only requires a brief two-step exposure of embryos/oocytes to the vitrification solution prior to plunging directly into liquid nitrogen. However, this method has traditionally used a specific type of vial as the sample carrier, which is not compatible with many laboratories which use plastic semen straws as their preferred storage system.

At the MRC we have developed an aluminium cooling plate for vitrification in straws. The cooling plate ensures stable refrigeration at 0°C during the equilibration process. In the experiments reported here, 2-cell embryos from C57BL/6N Tac mice were used. For comparison, embryos were vitrified in straws (n=3) and vials (n=3) using DAP213 as vitrification solution. The viability of vitrified embryos was compared in terms of post-warming survival rate, as well as their in vitro and in vivo developmental rates. The results demonstrated that there is no significant difference between straws and vials in survival rate (97.14 ± 2.86 vs 98.10 ± 0.95), development to blastocyst (94.74 ± 2.90 vs 84.48 ± 6.16) and birth rate (43.33 vs 46.67). These results indicated that the DAP213 vitrification procedure can be adapted for use with straws which are cheap to buy and compatible with space saving bulk tank storage systems. This method can also be used for oocytes vitrification.

Keywords: vitrification, mice, embryo, oocyte

**P2: Derivation of Extra-Embryonic Endoderm Cells from Preimplantation Pig Blastocysts**

Chi-Hun Park1,2, Anne Powell2, David M. Donovan2, Bhanu P. Telugu1,2  
1Department of Animal and Avian Sciences, University of Maryland, College Park, MD, USA; 2Animal Bioscience and Biotechnology Laboratory, USDA-ARS, Beltsville, MD, USA

In the fertilized mammalian embryo, the development of blastocyst marks the first visual cell fate decision. In the blastocyst, the precursor cells for placental extraembryonic membranes called the trophectoderm (TE) line the outside of the embryo, whereas, the cells on the inside aptly called the inner cell mass (ICM) give rise to the fetus proper. Following this first lineage differentiation event, a group of cells from the ICM also give rise to the primitive endoderm cells (PrE) that line the inside of the fluid filled blastocoel cavity forming the yolk sac. In culture, the blastocyst stage embryos give rise to characteristic lines derived from the TE, ICM and PrE.
Here we describe the establishment and characterization of extraembryonic endoderm (XEN) cell lines derived from PrE of day 7 pre-implantation pig blastocysts. In vitro generated porcine D7 blastocysts were explanted onto irradiated mouse embryonic fibroblasts in DMEM medium supplemented with 15% fetal calf serum (FCS), leukemia inhibitory factor (LIF), and basic fibroblast growth factor (bFGF). After 2 days of culture, three types of cell outgrowths as evidenced by morphology and the expression of lineage-specific markers were identified. A cluster of cells with compact morphology and likely derived from the epiblast (Epi) expressed key pluripotent markers (e.g. NANOG, and SOX2), whereas the other two cell types that exhibited a cobble-stone morphology expressed markers of TE-specific genes (CDX2) or PrE-(GATA4 and GATA6). Following initial establishment, Epi and TE cells have mostly disappeared during further subculture, whereas XEN cell colonies expressing GATA factors grew rapidly and were continuously cultured for over 30 passages. These cells showed high level of SALL4 expression, which contributes to the regulation of stemness of XEN cells, but not AFP, which is a visceral endoderm marker. Its proliferation was dependent on feeder cells with either LIF or FGF signal molecules. In summary, we demonstrated that the XEN cell lines from day 7 pig blastocysts can be reproducibly derived and the morphological and molecular characteristics of these cells are similar to mouse XEN cells. However, further studies are needed to determine whether pig XEN cells fulfill the requirement of typical XEN cells features.

**P3: Disruption of CFTR Gene in Sheep Fibroblast Cells Using CRISPR/Cas9 Approach**

Zhiqiang Fan¹, Zhongde Wang¹, Iuri V. Périssé¹, Min Yang¹, Misha Regouski¹, Ann Harris², Kenneth L. White¹, Irina A. Polejaeva¹

¹ Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322, USA; ² Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL 60614, USA.

Cystic Fibrosis (CF) is a common life-shortening, autosomal recessive disease in Caucasian populations that affects approximately 1 of every 2500 newborns. The CF gene was identified as the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) encoding a conductance chloride anion channel. Sequence analysis of CFTR shows 90% similarity at the nucleic acid level and 95% at the polypeptide level between sheep and human. A sheep model of CF is likely to be beneficial in investigating CF pathogenesis and furthering pharmacological approaches to the treatment of CF. Here, we report the successful application of the CRISPR/Cas9 system to disrupt CFTR in sheep fibroblast cells, which can be used for the production of cloned sheep by Somatic Cell Nuclear Transfer (SCNT). We designed 5 single-guide RNAs (sgRNAs) specific for CFTR: 2 for exon 2 and 3 for exon 11. The targeting vectors were constructed by using pX330 plasmid and transfected into 2 x 10⁵ male fetal fibroblast cells. The gene mutation efficiency of each targeting vector was determined by PCR/RFLP assay 3 days post transfection. Our results showed that 2 sgRNAs, 5’- GAAAGGATACAGACAGCGCT – 3’ for exon 2 and 5’–GGGAGAATTGGAACCTTCAG – 3’ for exon 11, were highly efficient in directing Cas9 to generate targeted cleavages in CFTR, with mutation efficiencies of 18.5 % and 40.9 %, respectively. Sequencing analysis of PCR products showed that typical nucleotide insertions and deletions, caused by repairing double-strand DNA breaks during the error-prone non-
homologous end joining process, were generated in each of the targeted CFTR loci. We also isolated single cell-derived sheep fetal fibroblast colonies by limiting dilution following transfection. The colony screening with the PCR/RFLP assay confirmed that we achieved targeted gene disruption in 10/51 (19.6%) colonies for exon 2 (5 colonies with biallelic and 5 with monoallelic CFTR gene disruption), and in 17/49 (34.7%) cell colonies for exon 11 (13 with biallelic and 4 with monoallelic). In conclusion, we demonstrate that CRISPR/Cas9 is a highly efficient system for generating targeted mutations in CFTR gene in sheep fetal fibroblasts. These cells will be subsequently used for SCNT to generate sheep models of CF.

**P4: DNA Methylation and Gene Expression Levels in Hypothalamus and Ovary of Capra hircus Across the Genome**

Stefano Frattini¹, Barbara Lazzari²,³, Emanuele Capra², Andrea Talenti¹, Beatrice Coizet¹, Stephanie D. McKay⁴, Alessandra Stella²,³, Giulio Pagnacco¹, Paola Crepaldi¹

¹Department of Veterinary Medicine, University of Milan, Milan, Italy; ²Institute of Agricultural Biology and Biotechnology, National Research Council UOS of Lodi, Lodi, Italy; ³PTP - Science Park, Lodi, Italy, ⁴Department of Animal & Veterinary Sciences University of Vermont, Burlington, VT, USA

Hypothalamus and ovary are two key organs of the female reproduction system of mammals. Their role in controlling ovulation is well known, but the epigenetic mechanisms behind it, such as DNA methylation, remain unclear. Goats are an important source of milk, meat and fiber, especially in developing countries. Despite this, among economically relevant livestock species, they are still poorly investigated from a genomic point of view. The aim of this work is to find a relation between expression levels and methylation levels, in order to understand how methylation peaks in different gene regions affect gene expression in goat hypothalamus and ovary. Genome-wide methylated CpG detection and gene expression analysis in hypothalamus and ovary of three adult Saanen goats were performed. Animals in the study were managed according to the existing European Directive 2010/63/EU on the protection of animals used for scientific purposes. For the methylome analysis DNA binding domain sequencing (MBD-seq) with enrichment of methylated DNA fragments was performed. Gene expression was evaluated by RNA-Seq analysis. Sequencing was accomplished with Hiseq 2000 Illumina. Around 23-37 million raw sequencing reads were generated from each sample for the methylome analysis. Methylation distribution was investigated in six different genomic regions: promoter, intron, exon, downstream of gene, distal and intergenic. Matching the methylation pattern in hypothalamus and ovaries in contrast to their transcriptome allowed the identification of genomic regions in which methylation peaks most affect gene expression. Hypothalamus showed a highly significant negative correlation (P<0.001) between methylation peaks in promoter and in downstream regions and gene expression. A positive correlation (P<0.001) was observed within exons. Conversely, ovary did not show any significant consistent correlation between gene expression and methylation. This work provides evidence for a clearer understanding of the epigenetic mechanisms underlying gene regulation. A more accurate annotation of the goat genome will be necessary for a deeper insight in the role of DNA methylation in gene expression in Capra hircus, a candidate model species for other mammals.
**P5: Effect of Neuromedin U on Pig Immune Regulation**

Xiang Li, Xiaoliang Li, Wenna Qiao, Zhiyu Ma, Juan Su, Zhihai Lei
College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu, P.R. China

Neuromedin U (NMU) is a conserved mammalian neuropeptide discovered in the 1980s, and found in two forms, NMU-25 and NMU-8. Wide distribution of NMU in animal organs suggests that NMU is involved in multiple physiological functions, including immune regulation. However, the role of NMU in pig immune regulation has not been reported. To study the effect of NMU on pig immune regulation, we cloned and detected the expression of NMU and its receptors in pig lymphatic organs and immune cells. We also investigated the effect of NMU on cytokine secretion after injection of (0, 5, 15, 45 nmol) NMU into the intracerebral ventricle (i.c.v) of 16 pigs (n=4 for each group), and the effect of (0.1~1000 nM) NMU on cytokine secretion in cultured dendritic cells and natural kill (NK) cells using ELISA and RIA methods. The results were as follows: 1) NMU and its receptors were expressed in lymphatic organs, cultured dendritic cells and NK cells. 2) NMU stimulated IL-1β, IL-6, IL-8, TNF-α and IL-10 secretion (P<0.05) post-injection in a time- and dose-dependent manner, compared with the control group. 3) NMU increased IL-8, IL-6 and IL-13 secretion and reduced IL-10 secretion (P<0.05) in cultured dendritic cells. 4) NMU enhanced the killing activity of cultured NK cells, stimulated IFN-γ secretion and inhibited IL-10 secretion (P<0.05) in NK cells in a time- and dose-dependent manner. This study suggests that NMU has the role in pig immune regulation through its effect on cytokine secretion and increasing killing activity of NK cells. Key words: NMU, Pig, NK cell, Dendritic cell, cytokine, immune regulation

**P6: Effect of Unbalanced Pedigree on Genetic Evaluation in Tunisian Holstein Cows**

Yosra Ressaissi¹, M. Ben Hamouda²
¹Département des Sciences Animales, Institut National Agronomique de Tunisie, 43 Avenue Charle Nicolle 1082-Tunis-Mahrajène-Tunisie. ²Institut National de recherches agronomiques de Tunisie, Rue Hédi Karray-2049 Ariana Tunis.

The pedigree quality was tested on the genetic evaluation process using 3 files. The first file was the original one which included the whole cows from which two extra pedigrees were generated: a file with only cows whose mothers are known and a file with only cows who’s both parents are known. A uni-trait animal model was fitted to 305-day dairy records (MY305) and counting on the restricted maximum likelihood procedure, the construction of the entire relationship matrix between cows and their ancestors was considered. The correlations of Pearson (r) and the distribution of the genetic deviation by flocks were made to appreciate relationship between the breeding values and to estimate genetic diversity according to the available genealogical information. Heritabilities were around 0.15 and 0.39 and genetic correlations have varied from 0.82 to 0.94. Using the balanced pedigrees, the genetic parameters were ameliorated and the coefficients of correlation were all positive while they were found to be higher between the predicted values using the balanced pedigrees. The distribution of the genetic variability was different according to the used pedigree. The heterogeneity in animal identification generates an
unbalanced pedigree of poor quality which penalizes the appropriate combination of the performances with the genealogical information. Consequently, the relevance of genetic evaluation and the estimation of genetic variability will be affected.

**P7: Establish of Immortalized Cell Line for Improving the Production Efficiency of the Disease-model Pigs**

Seon-Ung Hwang$^{1,2}$, Junchul David Yoon$^{1,2}$, Kiyoun Eun$^3$, Hyunggee Kim$^3,*$ and Sang-Hwan Hyun$^{1,2,*}$

$^1$Laboratory of Veterinary Embryology and Biotechnology, Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea; $^2$Institute of Stem Cell & Regenerative Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Republic of Korea; $^3$Department of Biotechnology, School of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea.

Production efficiency of disease-model pigs using somatic cell nuclear transfer (SCNT) is very low. It is probably because of senescence and the characteristics of the transgene. These make limitations in a pregnancy and a production of disease-model pigs. In this study, we introduce SV40LT oncogene into the fibroblast cells in order to establish immortalized transgenic cell line for producing the disease-model pigs. As a result, SV40LT cell lines showed that, even though a high passage, the normal cell morphology was observed with active cell division. We then evaluated the effect of SV40LT oncogene on transgenic SCNT embryo development. The cleavage rates (73.8±4.0 and 48.6±2.4 in the normal and SV40LT group, respectively; P<0.05) and blastocyst formation rates (19.5±1.2 and 5.6±1.8 in the normal and SV40LT group, respectively; P<0.05) of transgenic SCNT embryos was significantly lower than the case of using normal cells. In conclusion, the SV40LT inserted cell line showed active proliferation in the fibroblast state. However, that is not suitable for somatic cell nuclear transfer. To overcome this, the introduction of the induction system in cell lines, such as ecdysone-inducible gene switch, tetracycline-inducible system and Gal4/UAS system, is required to increase the production efficiency of the disease-model pigs. Keywords: Porcine, SCNT, SV40LT

This work was supported, in part, by a grant from the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011288, PJ011077)", and the "Ministry of Trade, Industry & Energy(MOTIE), Korea Institute for Advancement of Technology(KIAT) through the Leading Industry Development for Economic Region (Project No. R0004357)", Republic of Korea.
P8: Expansion and Differentiation of Isolated Porcine GFAP-CreERT2 Neural Stem Cells Using Neurosphere Assay

Eunhye Kim¹, Hyunggee Kim² and Sang-Hwan Hyun¹,*
¹Institute for Stem Cell & Regenerative Medicine (ISCRM), Chungbuk National University & Laboratory of Veterinary Embryology and Biotechnology (VETEMBIO), College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea; ²School of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea

In this study, we used the neural stem cells (pGFAP-CreERT2-NSCs) from the transgenic piglet with expression of CreERT2, a fusion protein of the DNA recombinase Cre and mutated ligand-binding domain of the human estrogen receptor, under the control of the GFAP promoter. The primary culture from tissue of porcine CreERT2 brain led to floating spherical masses of cells and quantitative analysis indicated a yield of 2.50±0.44 primary spheres per 1,000 viable cells from the neocortex, versus 12.92±1.67 primary spheres per 1,000 viable cells from the periventricular region (PVR) including subventricular zone (SVZ). Secondary spheres (6.67±1.10 spheres from neocortex versus 23.08±1.96 spheres from PVR cells) were formed from primary spheres at 10 days after passage. Tertiary spheres (8.42±0.99 spheres from neocortex versus 23.08±1.91 spheres from PVR cells) could also be obtained after a second passage indicating that they were proliferating in vitro. The CreERT2-NSCs showed normal 36+XY karyotype with all analyzed metaphases free of any discernable cytogenetic abnormalities and representative NSC markers such as NESTIN, SOX2 and VIMENTIN. After differentiation, we were able to obtain populations of astrocytes and neurons expressing GFAP and TUJ1, respectively. Although further studies will be needed, we developed a novel platform for expansion and neural commitment of the isolated GFAP promoter-driven CreERT2-NSCs which would be considered a useful tool for study of central nervous system diseases.

This work was supported, in part, by a grant from the “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011288, PJ011077)”, the Ministry of Trade, Industry & Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through the Leading Industry Development for Economic Region (Project No. R0004357)“ and “Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Advanced Production Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Grant number: 115103-02)”, Republic of Korea.
P9: Four Years of Editing the Livestock Genome

Chris Proudfoot1, Simon G Lillico1, Christine Burkard1, Wenfang Tan1, Maeve Ballantyne1, Claire Neil1, Scott C. Fahrenkrug2, Fyodor D. Urnov3, Alan J. Mileham4, Alan L. Archibald1 & C. Bruce A. Whitelaw4.

1 The Roslin Institute and R(D)SVS, University of Edinburgh, Easter Bush Campus, Edinburgh, EH25 9RG, UK; 2 Recombinetics Inc, 2575 University Ave. West, Suite 100, Saint Paul, MN 55108, USA; 3 Sangamo BioSciences, Point Richmond Tech Center, 501 Canal Boulevard, Suite A100, Richmond, CA 94804, USA; 4 Genus plc, 1525 River Road, DeForest, WI 53532, USA.

Recent advances in genome engineering have produced a range of tools that allow the precise modification of livestock genomes. Microinjected directly into the newly fertilised zygote, genome editor reagents are proving to be a very effective methodology for inducing changes in the DNA sequence at their target sites, opening up a multitude of options for genome manipulation. This ability to modify the genome, without the necessity of transgene insertion, allows the production of animals with designer traits such as improved disease resistance, enhanced production values or as models of human disease. We present data detailing our experiences with TALENs, ZFNs and CRISPR/Cas9 to induce NHEJ, HDR or deletion events in either sheep or pigs.

P10: Identification of Transgene Insertion Sites by Target Selection and Next-Generation Sequencing

Aaron J. Thomas1,2, Rousselene Jones1, Misha Regouski2, Irina Polejaeva2, Christopher J. Davies1,2

1 Center for Integrated BioSystems, Utah State University, Logan, UT, USA; 2 Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, USA

Atrial fibrillation is a serious cardiac arrhythmia for which better large animal models are needed. A transgenic goat expressing constitutively active Transforming Growth Factor-β (TGF-β) in the heart was created by random insertion of a construct containing the mouse α-Myosin Heavy Chain promoter driving expression of a constitutively active human TGF-β gene. Subsequent breeding of this female goat and clones of the founder animal produced three male offspring that tested positive for the transgene. To identify the transgene insertion site(s), we constructed sequencing libraries from genomic DNA of these goats and subjected them to target selection using RNA baits made from the transgene construct. The resulting libraries were then sequenced on the 454 GS FLX+. The sequence reads were aligned to the goat genome and to the transgene construct to identify those reads that had both goat genomic sequence and insert sequence. Two insertion sites were identified: one in the RASAL2 gene on chromosome 16 and one in the ULK4 gene on chromosome 22. Reads from the insertion site on chromosome 16 all mapped to only one end of the insertion site whereas reads from both ends of the insertion site on chromosome 22 were found. This is an effective method to find the genomic location of transgenes inserted by random insertion or to find off-target insertion events when targeted
insertion methods are used. The insertion site sequence information was used to design PCR primers that can be used to screen offspring of the clones to determine whether they carry one or both chromosomes with the TGF-β transgene.

**P11: Impact of Histone Acetylation Status on the In Vitro Development of Porcine Transgenic Cloned Embryos**

Biping Luo¹,², Shiqiang Ju¹, Rong Rui¹
¹ College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China; ² College of Animal Science & Technology, Jinling Institute of Technology, Nanjing, Jiangsu 210038, China

Transgenic cloning is a modern biotechnology combined transgenesis with nuclear transfer. Around 20 years have been passed after the birth of that famous cloned sheep “Dolly”. The somatic cell nuclear transfer used for the production of Dolly enables it possible to produce transgenic pigs. However, the efficiency to produce transgenic cloned pigs is at low level to date. Many factors impact on the development of transgenic cloned embryos, including epigenetic modifications. The purpose of this study was to investigate the effects of histone deacetylase (HDAC) inhibitor trichostatin A (TSA) on transgene expression and development of porcine transgenic cloned embryos, specifically, focusing on effects derived from TSA-treated donor cells or TSA-treated reconstructed embryos. The results showed that TSA treatment on reconstructed embryos modified the acetylation status, which significantly improved the development of porcine somatic cell nuclear transfer (SCNT) embryos in vitro, but not donor cells. Furthermore, the treatment of reconstructed embryos with TSA enhanced expression of the pluripotency-related gene POU5F1 and stimulated expression of the anti-apoptotic gene Bcl-2. Enhanced green fluorescent protein (EGFP) mRNA expression of every group dropped drastically from donor cells to blastocysts. Interestingly, TSA is likely to prevent a decline in EGFP expression in nuclear reprogramming of porcine SCNT embryos. However, DNA hypomethylation induced by modified histone acetylation of donor cells treated with TSA was significantly more effective to increase EGFP expression in SCNT blastocysts. In conclusion, acetylation status of both donor cells and reconstructed embryos modified by TSA treatment increased transgene expression and improved nuclear reprogramming and developmental potential of porcine transgenic SCNT embryos.

Keywords: acetylation status; development; transgene expression; TSA; EGFP; SCNT; pig
**P12: Integration of Molecular Imaging and Transgenesis for Developing Large Animal Models**

Liaohai Chen¹, Irina Polejaeva², and Zhongde Wang²
¹Department of Surgery, University of Illinois at Chicago, ²Animal, Dairy, and Veterinary Sciences Department, Utah State University, Logan, UT

Humane use of large animal models plays a critical role in understanding disease etiology and developing therapeutics. With the significant advances in gene targeting and somatic cell nuclear transfer, it is now feasible to produce a transgenic large animal with a latent transgene(s) embedded in its genome. However, large animals have a longer lifespan, and the impact of activated transgene(s), as well as the appearance of disease phenotype, may take a long time to manifest. The integration of molecular imaging with transgenesis enables the reveal of activation efficiencies, when, where, and the expression level of the transgene product(s). Otherwise, questions on whether the transgene is activated in the target organ and/or whether the transgene functionally contribute to the symptom/disease phenotype will require invasive surgical procedures or sacrifice of the animals, which will greatly hinder the development of large animal models. In addition, the ability to evaluate pharmacologic parameters through imaging on large animals will significantly lower the expense and the numbers of animals needed for the pre-clinical studies. Recently, we used somatic cell nuclear transfer (SCNT) method to develop mutated human K-ras transgenic goats as a potential large animal tumor model. Newborn kids have been confirmed to bear activable K-ras oncogene (K-rasG12D) and herpes simplex viral thymidine kinase HSV-tk gene, a well-established reporter gene. The presence of the report gene will enable imaging the effectiveness of K-ras activation, K-ras location/expression level upon activation in transgenic goats by Positron Emission Tomography (PET). In vitro experiments have confirmed the transformation from normal cells to tumor cells once the transgenes were activated. A clinical PET-CT system is being installed at USU.

**P13: In Vivo Ovarian Germ Cell Transgenesis**

Rhett L. Peterson, Kate C. Parkinson and Jeffrey B. Mason
Department of Animal, Dairy and Veterinary Sciences, Center for Integrated BioSystems, School of Veterinary Medicine, Utah State University, Logan, UT, USA.

In somatic gene transfer, the recipient's pattern of gene expression is changed, but the change is not passed on to the next generation. In germline gene transfer, the germline cells are changed with the goal of passing on the changes to offspring. We are currently investigating in vivo gene transfer to ovarian tissues (somatic and germline) using recombinant adeno-associated viral vectors. Our objectives are to 1) alter ovarian gene expression in vivo to modify the influence of the ovaries on the physiology of the recipient and 2) to use this technology to produce germline-transmissible changes in livestock species. What makes this work unique is that we are altering genetic influence focally (ovaries) in an otherwise normal, adult animal and these changes will be germline-transmissible. Adult, six-month-old female CBA/J mice had their ovaries surgically exposed to a eGFP-containing recombinant adeno-associated virus. Four weeks postoperatively, the females were induced to ovulate and the entire reproductive tract was collected. Ovaries were
routinely processed for histology using an anti-eGFP antibody. Ovulated oocytes were evaluated for eGFP expression at the time of collection. Ovulated oocytes and histological ovary sections both displayed evidence of eGFP transgene expression. Adeno-associated viral vectors normally remain episomal in transduced cells. However, transduced oocytes, upon fertilization will undergo meiosis exposing the 'normally episomal' transgene to meiotic recombination, similar to the process that allows incorporation of genes using oocyte/zygote microinjection. Future work will include in vitro fertilization of transduced oocytes, breeding of recombinant adeno-associated viral vector-exposed female mice and intra-ovarian transduction of ovaries in sheep.

**P14: Large Exotic Animal Models for Heritable Neurological Disease and Cancer**

Brian W. Davis, Ph.D.
Cancer Genetics and Comparative Genomics Branch, National Human Genome Research Institute, National Institutes of Health; Bethesda, Maryland, USA.

The expanding field of high throughput genomics has allowed the exploration of other model system for the identification of loci implicated in disease. Natural models for the study of genetic conditions are underway in felines and canines, as well as agricultural species. The population structure and veterinary surveillance of these organisms under human care lend themselves very well to understanding the mechanisms that underlie common conditions shared with humans. Recently, using RNAseq, our work has shown that canine transitional cell carcinoma, which has orders of magnitude higher occurrence of in certain canine breeds, shares a common somatic mutation (BRAF V600E) with several human cancers. An extension of this work is being explored for two conditions in exotic cats, which share similar population bottlenecks and veterinary surveillance as companion animals. In addition, their extended age in captivity (often twice that of the wild) aids in understanding disease associated with geriatry. Our data shows malignant melanoma is prevalent in the captive tiger population, but is enriched in certain breeding lines. The same is true for spinocerebellar ataxia in African lions, which is linked to hypovitaminosis A, regardless of diet. Using whole genome and transcriptome sequencing in collaboration with the Exotic Genome Repository (exoticgenome.org), we propose a model that incorporates populations maintained in zoological parks with generational health and breeding records as a proxy for human health. This not only provides mechanisms for testing human therapies in these species, as exemplified by canines, but allows the application of previously identified, targeted human therapies for use in these animals.
**P15: Management of Many Recessive Disorders in a Dairy Cattle Population Using Gene Editing and Sequential Mate Allocation**

John B. Cole  
Animal Genomics and Improvement Laboratory, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA

High-density single nucleotide polymorphism genotypes have recently been used to identify a number of novel recessive mutations that adversely affect fertility in dairy cattle, as well as to track conditions such as red coat color and polled. Recent findings suggest that the use of sequential mate allocation strategies that account for increases in genomic inbreeding and the economic impact of affected matings result in faster allele frequency changes than strategies which do not consider inbreeding and monetary losses. However, the effect of gene editing on selection programs also should be considered because gene editing has the potential to dramatically change rates of allele frequency change. A simulation program developed to evaluate dairy cattle breeding schemes was extended to include the use of CRISPR-Cas9, TALEN, and ZFN for gene editing. A hypothetical technology with a perfect success rate was used to establish an upper limit on attainable progress, and a case with no editing served as a baseline for comparison. The technologies differed in the rate of success of gene editing, as well as the success rate of the embryo transfer step, based on estimates in the literature. For purposes of this study the number of alleles edited had no effect on success rate. The two scenarios evaluated considered only the horned locus, or 12 recessive alleles currently segregating in the US Holstein population. The top 1, 5, or 10 % of bulls were edited each generation, and either no cows or the top 1 % of cows were edited. Cumulative genetic gain did not differ across scenarios because the elite animals in the population were the ones selected for genetic modification. Gene editing was very effective at reducing the frequency of the horned haplotype (increasing the frequency of polled animals in the population), and allele frequencies of the 12 recessives segregating in the US Holstein population decreased faster with editing than without. These results suggest that gene editing is an effective tool for reducing the rate of harmful alleles in a dairy cow population even if only a small proportion of elite animals are modified.

**P16: MedSwine: Engineering Swine Genome for Biomedical Models**

Bin Zang¹, John Garbe², Dan Carlson⁴, Scott Fahrenkrug³,⁴  
¹Scientific computation, University of Minnesota, 200 Union St, Minneapolis, MN 55455, USA, Minnesota Supercomputing Institute, University of Minnesota, 117 Pleasant St SE, Minneapolis, MN 55455, USA; ²Department of Animal Science, University of Minnesota, 1364 Eckles Ave, Saint Paul, MN 55108, USA; ³,⁴Recombinetics, Inc., 1246 University Avenue W, #301, Saint Paul, MN 55104, USA

Large Animals Better Resembles Human Disorders  
We firstly compared several model animals including mice, rats, zebrafish as well as pigs to find out the resemblance of human disorders with known disease-gene relationship. By measuring the proteome level similarity of gene sets that are related to different disorder types, we identified
disorders in human that can be better modelled with large animals, especially pigs, than murine models. We find that these disorders include human metabolic syndromes such as MODY, and certain cancer types, such as breast cancer and liver carcinoma.

Imputing Human Disease-causative Mutations in Pig Genome
We collected variations presented in human genome with clinical significance from OMIM curated disease associated genes, of which, 5,916 have been successfully annotated in current pig genome assembly. We used protein alignment of the corresponding orthologous gene pairs to annotate potential editing sites within the pig genome. Overall, we imputed 947 nonsynonymous stop gained deletions, leading to partial/complete loss of function mutations, as well as 4,330 missense mutations in human genome to have corresponding pig edit sites. This result enables us to edit the pig genome for the model of 2,484 human disorders with at least one confirmed associated SNP.

In silico Off-target Prediction of Editing Tools
Rise of genome editing tools such as CRISPRs and TALENs gave us power to easy editing the genome. With pre-computed nucleases, we identified 5,363 CRISPRs sit nearby a disease associated SNP site. Among all these CRISPRs 1,603 of them sits within 20bp range of the SNP, indicating a set of good target candidates for human disease model.

MedSwine as an Integrative Resource for Pig Models
We also present MedSwine, a one-stop resource for engineering the pig genome for human biomedical models. MedSwine provide a resource to share aforementioned human disease models within the context of the pig genome. It also allows users to navigate, inspect and select putative nucleases around target mutation sites. MedSwine is under active development with scheduled feature integration.

**P17: Molecular Basis of Atrial Fibrillation in TGF-β Transgenic Goats**

Misha Regouski¹, Dustin H. Liechty¹,², Arnaud Van Wettere¹,², Heloisa M. Rutigliano¹,², Irina A. Polejaeva¹

¹Department of Animal, Dairy, and Veterinary Sciences, Logan, UT ²School of Veterinary Medicine, Utah State University, Logan, UT

Atrial fibrillation (AF) is one of the most prevalent cardiac arrhythmias. Studies have shown associations between cardiac fibrosis and incidence of AF, however the underlying mechanisms of this relationship is poorly elucidated. Our group has developed the first transgenic goat model of cardiac fibrosis. These transgenic goats (with overexpression of human TGF-β1) provide a unique platform to study the mechanisms underlying AF. We hypothesize that genes associated with the TGF-β signaling pathway, fibronectin, and collagens will be upregulated in the heart tissue of transgenic goats, as well as inflammatory cytokines. Atrial and ventricular samples were collected from TGF-β1 transgenic goats (n=6) and wild-type controls (n=5). Transgenic animals included in this study expressed the transgene in a cardiac specific manner and exhibited increased atrial fibrosis and AF inducibility. Preliminary gene expression analysis was performed following standard Fluidigm protocol (Spurgeon et al., 2008). Genes analyzed included housekeeping controls, and those associated with TGF-β and fibrosis pathways, inflammatory mediators, and apoptosis. Immunohistochemistry was conducted in order to characterize
different leukocyte cell populations (CD2+, CD4+, CD8+, CD14+, CD21+, MHC-II+ and FoxP3+ cells). Imaging was done using a high-resolution AxioCam HRC digital camera and analyzed using AsioVision software. We expect to identify an increase in lymphocytes such as CD2+, CD4+ and CD8+ T cells and macrophages (CD14+ and MHC-II+) in the myocardium of transgenic goats when compared to their wild-type controls. In addition, histological sections stained with Masson’s trichrome stain and hematoxylin and eosin were analyzed. Within each section 10 randomly selected fields of view were photographed using an Olympus DP 26 digital microscope camera and CellSens digital imaging software (Olympus Corporation). Fibrosis quantification was determined by measuring percentage area occupied by blue staining (Masson’s trichrome stain) of total surface area using Photoshop CS6 (Adobe Systems). Cardiomyocyte diameter was assessed by measuring distance of the narrowest plane across the cell nucleus for 100 cells from each atrium and ventricle from control and transgenic goats. Data generated by these experiments will aid in the identification of the molecular basis of cardiac fibrosis and atrial fibrillation in order to facilitate better treatment options for patients with AF.

**P18: Novel Accessible Method to Produce Porcine Chimeric Embryo**

**Junchul David Yoon**¹, **Eunsong Lee**² and **Sang-Hwan Hyun**¹*

¹Institute for Stem Cell & Regenerative Medicine (ISCRM), Chungbuk National University, Cheongju, 28644, Republic of Korea, ²Laboratory of Theriogenology, College of Veterinary Medicine, Kangwon National University, Kangwon, Republic of Korea

Since the pluripotent stem cells (PSC) was discovered including embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC), the PSCs was look forward with the keenest anticipation to differentiate functioning organs in regenerative medicine. Recent decade, the studies for generation of functional organs have been advanced remarkably with underlying mechanisms for production of chimeric animals. However, the procedure of chimeric animal production is intricate and it became a major reason of low in vitro production efficiency. The purpose of this study is to investigate to establish the novel accessible method to produce porcine chimeric embryo. In first, we produced EGFP-marked porcine fibroblast, and then established the EGFP labeled porcine ES-like cells via somatic cells nuclear transfer (EGFP-ntESC). The produced ntESC were injected into 2-, 4-, and 8 cells stage of cleaved parthenogenesis embryo at day 2 under microscope with manipulators. We investigated chimeric embryo development, and counted total cell number and EGFP-labeled cell number from achieved blastocysts. Data were analyzed by ANOVA followed by Duncan using SPSS (Statistical Package for Social Science). After day 5 of in vitro chimeric embryo culture (IVC), ES cells injected on the 4- and 8 cells stage groups showed significantly higher total blastocyst (49.6% VS 63.9% and 65.6%, respectively) and expended blastocyst formation rate (30.2% VS 50.7% and 52.2%, respectively) compared with ES cell injected on the 2 cell stage group. Under epifluorescence (460 nm) analysis, the 8 cell groups showed significantly higher EGFP-ntESCs number and distribution in achieved blastocyst compared with 2 cell group (4.0 and 6.0% VS 1.8 and 3.1%), even though we could not find any significant differences on blastocyst total cell numbers (60.6, 73.8, and 87.6, respectively). In conclusion, ES cell injection on 8 cell stage is applicable to produce
porcine chimeric embryo. However, improvement of injected ES distribution in blastocyst required for effective production of in vitro chimeric embryo.

Keywords: porcine, Chimeric embryo, EGFP

Acknowledgement
This work was supported, in part, by a grant from the “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011288, PJ011077)” Rural Development Administration, the “Ministry of Trade, Industry & Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through the Leading Industry Development for Economic Region (Project No. R0004357)” and “Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Advanced Production Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (Grant number: 115103-02)”, Republic of Korea.

Corresponding author: shhyun@cbu.ac.kr

**P19: Reduced Gene Expression and Mitochondrial Function in Porcine Donor-fibroblasts Treated with CPI-613 or PS48 Does Not Negatively Impact Subsequent In Vitro Development After Somatic Cell Nuclear Transfer**

Bethany R. Mordhorst, Stephanie L. Murphy, Joshua A. Benne, Bethany K. Redel, Lee D. Spate, Kevin D. Wells, Jonathan A. Green, Randall S. Prather.

Department of Animal Science, University of Missouri, Columbia, MO, United States.

Early embryos exhibit characteristics of a Warburg Effect (WE)-like metabolism. A hallmark of the WE is predominate use of glycolysis for energy production as opposed to the tricarboxylic acid (TCA) cycle. In cancer cells increased signaling of the PI3K pathway is correlated with an increase in glucose metabolism consistent with the WE. PS48 stimulates the PI3K pathway and CPI-613 inhibits pyruvate dehydrogenase. Both compounds should decrease mitochondrial use of the TCA cycle and promote the PI3K pathway. The goal was to achieve a WE-like effect in donor-cells prior to nuclear transfer (NT) by treating day 35 porcine fetal fibroblasts for 7 days. Data were analyzed for main effect of drug via GLM procedure of SAS 9.4 (Cary, NC). JC-10 was used to determine the mitochondrial membrane potential (Δψm). The percentage of cells with high Δψm decreased (P <0.01) with 10µM PS48 (33.4%), CPI 100µM (1.76%), or the combination (MIX; 2.81%) compared to control (CON; 46.5 ±3.3%). Expression of mRNA was measured by qPCR ΔΔct method. CPI and PS48 decreased PKM M1 variant expression compared with control (P< 0.01). Treatments decrease Δψm and PKM M1 abundance, therefore metabolism of these potential donor cells may be more WE-like. Fibroblasts treated with CPI, PS48, and MIX were used for NT in enucleated oocytes. Cleavage and blastocyst percentages, blastocyst cell number, and TUNEL positive cell number were not augmented by donor-cell treatment (P >0.14). Drug treatments were tested in the culture of SCNT embryos derived from non-drug-treated fibroblasts. Blastocyst development was impacted by drug culture treatment (P= 0.05): PS48 and CON had higher percentages (43.3 and 41.2%) compared to CPI and MIX (33.6 and 32.7 ±2.9%). TUNEL positive cell number was increased in MIX embryos vs. other treatments (P =0.01; MIX =2.1 vs. ≤1.4 ±0.26 in other treatments). Zygotic cleavage rate and cell number within blastocysts were not altered with embryonic drug culture (P ≥ 0.07). To date, we
have 3 successful day-35 fetal collections resultant from embryo transfers of PS48 and CPI donor-treated clones. Future experiments will further investigate embryonic metabolic programming. Funded by Food for the 21st Century and NIH R01HD080636.

**P20: Reprogramming Bovine Fibroblasts to Pluripotency by Fusion to Mouse Embryonic Stem Cells**

Maria-Cristina Villafranca, Kaylyn R. Williams, Will H. Eyestone
Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061, USA

Embryonic stem cells (ESCs) are pluripotent cell lines derived from preimplantation embryos of mice, humans and non-human primates. Induced pluripotent cells (iPSCs) are generated from differentiated cells by ectopic expression of transcription factors found in ESCs, and are morphologically and functionally equivalent to ESCs. The bovine is an important agricultural species for which neither ESCs nor iPSCs exist. Traditional transcription-factor based reprogramming, even in the presence of reprogramming enhancers, is insufficient to reprogram bovine somatic cells to pluripotency. Mouse and human somatic nuclei can be reprogrammed to pluripotency when fused to mouse ESCs (mESCs), suggesting that mESC cytoplasm contains reprogramming factors. We hypothesize that mESCs will reprogram bovine somatic cells upon fusion and plan to use this model to identify additional/alternative reprogramming factors. We developed a cell fusion method using polyethylene glycol and dimethyl sulfoxide (DMSO). Additionally, we designed a method to detect and select heterotypic (i.e. mESC/somatic cell; heterokaryons) from homotypic fusions (i.e. same cell type fusion products; homokaryons). Bovine fibroblasts (BFs) were transduced with a polycistronic lentiviral vector harboring a CMV promoter-driven cyan fluorescent protein (CFP) and an Oct4 promoter-driven GFP reporter, as well as a puromycin selectable marker. We transduced mESCs with a lentiviral vector harboring a CMV promoter-driven monomeric red fluorescent protein (mRFP) reporter. With these tools in place, selected BFs and mESCs were fused and monitored for reprogramming by upregulation of Oct4-driven GFP expression. We observed expression of Oct4-driven GFP in cells expressing both CMV and mRFP. We plan to screen heterokaryons for differentially expressed genes using RNA-seq. To avoid lentiviral integrations, we designed an alternative method to label BFs and mESCs using fluorescently labeled antibodies. Upon fusion, heterokaryons were labeled with anti-bovine CD44 and anti-mouse SSEA-1 antibodies. Heterokaryons positive for both markers were manually selected using a micromanipulator. We are currently performing the necessary steps for RNA-seq using RNA from <20 heterokaryons. Future research will involve testing candidate reprogramming genes for reprogramming activity by forced expression in BFs. We plan to further characterize the cells obtained with our new set of candidate factors by establishing comparisons with cells reprogrammed with currently known reprogramming factors.
**P21: Targeted Gene Knock-in by CRISPR/Cas Ribonucleoproteins in Porcine Zygotes**

Ki-Eun Park¹,²,³, Anne Powell², Shelley E.S. Sandmaier¹,², Chan-Mi Kim¹,², Alan Mileham⁴, David M. Donovan², and Bhanu P. Telugu¹,²,³

¹ University of Maryland, Animal and Avian Sciences, College Park, MD, USA; ² USDA-ARS, Animal Bioscience and Biotechnology Laboratory, Beltsville, MD, USA; ³ Livestock Genomix, Reisterstown, USA; ⁴ Genus plc, DeForest, WI, USA

The domestic pig is an important “dual purpose” animal model for agricultural and biomedical applications. There is an emerging consensus in the biomedical community that there is a requirement for large animal models such as pigs that can either serve as an alternative, or complement investigations from the mouse. However, the use of pig has not proven popular to date due in part to technical difficulties and time required in generating desired genetic modifications. In this regard, the ability to directly modify the genome in the zygote and generate edited animals is highly desirable. This report demonstrates for the first time, the generation of gene knock-in animals by direct injection of Cas9 ribonucleoprotein complex and short stretches of DNA sequences into porcine zygotes. The Cas9 protein from Streptococcus pyogenes was precomplexed with a single guide RNA targeting downstream of the ubiquitously expressed COL1A gene, and co-injected with a single-stranded repair template into porcine zygotes. Using this approach, pigs that carry pseudo attP sites within the COL1A locus to enable phiC31 integrase mediated introduction of transgenes have been generated. This new route for genome engineering in pigs via zygote injection should greatly enhance applications in both agriculture and biomedicine.

**P22: TCET: A Novel Non-surgical Embryo Transfer Device**

Ruonan Liu¹, Yanping Miao¹, Xinchong Duan¹, Mo Guan², Xiangyun Li¹

¹ College of Animal Science and Technology, Agricultural University of Hebei, Baoding, Hebei 071000, China. ² Medical Research Council, Mary Lyon Centre, Harwell Campus, Oxfordshire, OX11 0RD, UK

Although non-surgical transfer of embryos in mice has many advantages over a surgical method, the variable success of non-surgical transfer has hampered its acceptance and use. In our studies, we discovered that the uterine injury and embryo loss during the procedure are the main causes of the variable success for non-surgical transfer. We found that the material and shape of the transfer catheter, the implantation-related factors, the volume of transfer medium and the techniques of transfer significantly affected embryo implantation. Therefore, we developed a novel non-surgical device - TCET (Transcervical Embryo Transfer, Elim Springs Biotech Ltd, UK). The device comprises of an arched, thin, soft tip catheter with blunt ends, which will avoid or minimize the risk of scratching the endometrium or puncturing the uterine horn during the procedure, and the right length of the catheter tip can improve the delivery of the embryos in the best position in the uterus for successful implantation. We also refined the transfer techniques and the component and volume of transfer medium, which significantly reduces the uterine injury and embryo loss and improves the embryo implantation. In these experiments reported
here, 62 blastocysts from natural cycle CD1 mice were non-surgically transferred into the uteri of 8 CD1 recipient females (2.5d). As the control, 116 embryos were surgically transferred into the uteri of 15 CD1 recipient females (2.5d). The results showed that 100% recipient females (8/8) got pregnant and gave birth to 51 live pups (82.2%) from TCET transfer. The results from the control group were: 93.3% pregnancy rate (15/16) and 70.6% birth rate (82/116). Compared with surgical embryo transfer, the procedure of TCET transfer is much simpler, quicker, easier, markedly less traumatic and less stressful to the recipient females. In Summary, we established a novel and efficient non-surgical embryo transfer using TCET here, which could be an alternative to surgical embryo transfer. In addition, this non-surgical embryo transfer technique in mice establishes an animal model for the study of rats, cattle, other large animals and human embryo transfer techniques.

**P23: The International Society for Transgenic Technologies – Large Animal/Non-Rodent Initiative**

Jan Parker-Thornburg¹, Bruce Whitelaw², Martina Crispo³

¹ ISTT, Inc. President, Department of Genetics, MD Anderson Cancer Center, Houston TX USA; ² The Roslin Institute, University of Edinburgh, Midlothian UK; ³ ISTT, Inc. Board Member, Transgenic and Experiment Animal Unit, Institut Pasteur, Montevideo, UR

The International Society for Transgenic Technologies, Inc. (ISTT, Inc.) was founded in January 2006 in Spain, and was reincorporated in the USA in May, 2013. The Society seeks to foster communication and sharing of technology, to enhance scientific research, to advance the field of animal transgenesis, particularly as it applies to useful experimental models in biology, medicine and biotechnology, and to represent the interest of scientists, technicians, and graduate students working in the field of transgenic technologies. The ISTT sponsors a series of periodic international meetings known as the Transgenic Technology (TT) Meetings. At TT2016, held in Prague CZ (March 2016), the ISTT instituted a Large Animal/non-Rodent initiative, to be headed by Bruce Whitelaw (Roslin Institute) and Martina Crispo (Institute Pasteur, Uruguay, ISTT Board member). The ISTT Board encourages all investigators working in non-rodent models to join and contribute to this effort.
**P24: To Make an Alzheimer’s Disease (AD) Sheep Model Using CRISPR Genome Editing**

Jun Liu¹, Jen M. Kelly², Clive J. McLaughlan³, C. Simon Bawden³, Susanne J. Reid⁴, Russell G. Snell⁴, Paul J. Verma¹,²

¹ Stem Cell and Genetic Engineering Group, Department of Materials Engineering, Monash University, Clayton, VIC, Australia; ² Reproductive Biology, Turretfield Research Centre, South Australian Research and Development Institute, Rosedale, SA, Australia; ³ Molecular Biology Laboratory, South Australian Research and Development Institute, Roseworthy, SA, Australia; ⁴ Centre for Brain Research, School of Biological Sciences, University of Auckland, Auckland, New Zealand

Objective: Presenilin 1, encoded by the PSEN1 gene, is one of four core proteins in the gamma-secretase complex, which mediates regulated proteolysis of several proteins in the cell, including the amyloid precursor protein (APP). Gamma-secretase activity generates specific beta-amyloid 42) accumulates to form the intraneuronal plaques associated with AD. A simple glutamic acid-to-alanine mutation at codon 280 (E280A) in PSEN1 alters the activity of gamma-secretase and results in increased levels of the toxic 42 peptide. To attempt creation of AD sheep, the project aims to replace the equivalent glutamic acid codon in the sheep PSEN1 protein (E277) with an alanine codon.

Methods: A pair of 20bp gRNA was designed using online CRISPR design tools. The gRNAs were annealed and sub-cloned into pSpCas9(BB) and lentiCRISPRv2 backbone vectors using Zhang’s lab protocols. Two HDR oligos of 202bp and 194pb, respectively, were designed and synthesized by IDT.

Results:

Functionality of the CRISPR in HEK293T cells: Reporter vectors were constructed using GeneArt genomic cleavage selection kit. Using lipofactamine 3000 transfection reagents, the reporter and pSpCas9(BB)-sgRNA vectors were transfected into HEK293T cells. 20 h post transfection, positive OFP cells were detected under a fluorescence microscope. FACS analysis showed that 25.6% of the cells were OFP positive.

Functionality of the CRISPR in sheep iPS cells: lentiCRISPRv2-sgRNA#2 was co-transfected with packaging plasmids pMD2.D and psPAX2 into HEK293T cells to produce lentiviral particles, which were then transduced into sheep iPS cells using Fugene 6 reagent according to manufacturer’s instructions. 48 h later, the cells were cultured in medium supplemented with 100 ng/ml puromycin. Genomic DNA was extracted from the surviving colonies. T7EI assay showed two additional digested products as well as full-length PCR products. Subsequent sequencing analyses showed that random-sized Indel mutations were created at the desired locus in the iPS cell colonies.

Generation of targeted sheep mutants by embryo injection of CRISPR mRNA: A mixture of Cas9 mRNA, sgRNA, and HDR single-strand oligo were injected into the sheep one-cell stage embryos. The injected embryos were cultured in vitro for blastocyst formation. Genomic DNA was extracted from the blastocysts and subjected to genotyping. Out of 100 sequenced blastocysts, 2 embryos showed the HDR mutation in the genome.
Index
“S” denotes speakers, “P” denotes posters
Ayares, David, S10
Carlson, Dan, S2
Chen, Liaohai, P12
Cockett, Noelle, S19
Cole, John, P15
Cutler, Michael, S13
Davis, Brian, P14
Desaulniers, Amy, S10
Fan, Zhiqiang, P3
Frattini, Stefano, P4
Guan, Mo, P1
Harris, Ann, S18
Hwang, Seon-Ung, S11, P7
Kim, Eunhye, P8
Laible, Goetz, S3
Li, Xiang, P5
Liu, Jun, P24
Liu, Ruonan, P22
Luo, Biping, P11
Mason, Jeff, P13
Mordhorst, Bethany, P19
Park, Chi-Hun, P2
Park, Ki-Eun, P21
Parker-Thornburg, Jan, P23
Piedrahita, Jorge, S6
Polejaeva, Irina, S8
Prather, Randy, S9
Proudfoot, Chris, P9
Ranjan, Ravi, S8
Regouski, Misha, P17
Ressaissi, Yosra, P6
Roberts, Michael, S1
Rogers, Chris, S14
Sheets, Timothy, S15
Sullivan, Eddie, S7
Telugu, Bhanu, S4
Thomas, Aaron, P10
Villafranca, Maria-Christina, P20
Wells, Kevin, S5
Wolf, Eckhard, S17
Yang, Min, S16
Yoon, Junchul David, P18
Zang, Bin, P16
Our transgenic goats are the first of their kind, and may help doctors understand and treat the most common heart arrhythmia in humans. Learn more about them in Session 3 of the Large Animal Genetic Engineering Summit and in the August 2016 issue of the *Journal of Cardiovascular Electrophysiology.*
NAVIGATE
COLLABORATE
SUCCEED

Equity-free Federal Research Dollars for Small Business Technology
If you are an American-owned and independently operated for-profit company with fewer than 500 employees, you may qualify for research and development grants with no repayment, equity dilution or IP forfeiture.

USTAR’s SBIR-STTR Assistance Center Services:
Never miss a solicitation! Ask about our Search Service!

- Consultation
- Search
- Preparation
- Writing
- Editing
- Submission

To work with USTAR’s SSAC, your company must have a Utah Business License.

CONTACT:
USTAR’s SBIR-STTR Assistance Center
801.957.5249
utahsbir-sttrcenter@utah.gov
ustar.org
Located at and in partnership with:
Salt Lake Community College-Miller Campus
9690 South 300 West • Sandy, UT 84070
We're changing the landscape of human health.

By leveraging the world's first large animal platform to produce human antibodies, we have the potential to transform the treatment and prevention of disease.

Yorktown Technologies, L.P.

glofish.com

glofish.com

transova.com

simplot.com/animalsciences

NOTES