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In Memoriam: John K. Critser (1953-2011)

Dr. John K. Critser, Gilbreath-McLorn Professor for Comparative Medicine at the University of Missouri College of Veterinary Medicine (1953-2011).

Dr. Critser received a BA in biology and philosophy from Ripon College in Ripon, Wisconsin, a MS in Veterinary Science and a PhD in Animal Science from the University of Madison, WI. John's first position was as Director of Andrology/Cryobiology at Methodist Hospital of Indiana. His first faculty appointment was in the Department of Physiology/Biophysics at Indiana University's School of Medicine. He went on to also have appointments at the Purdue School of Veterinary Medicine. John came to the University of Missouri in 2001 when he was recruited as the Gilbreath-McLorn Professor for Comparative Medicine at the MU College of Veterinary Medicine.

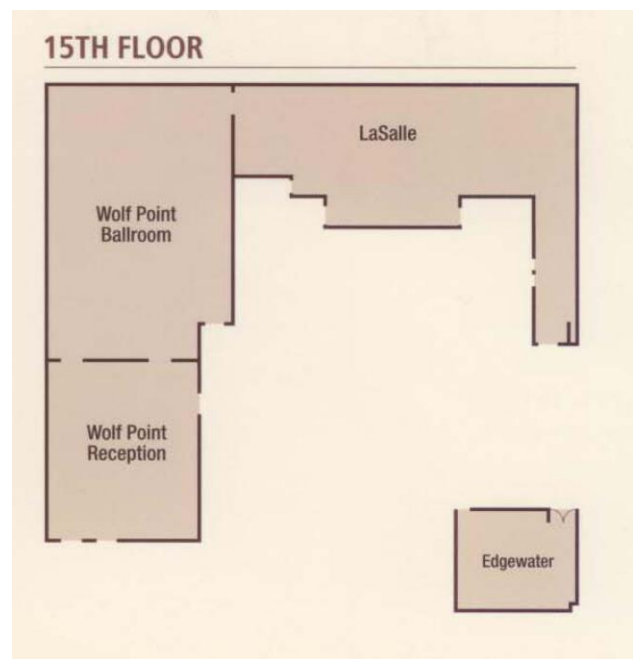
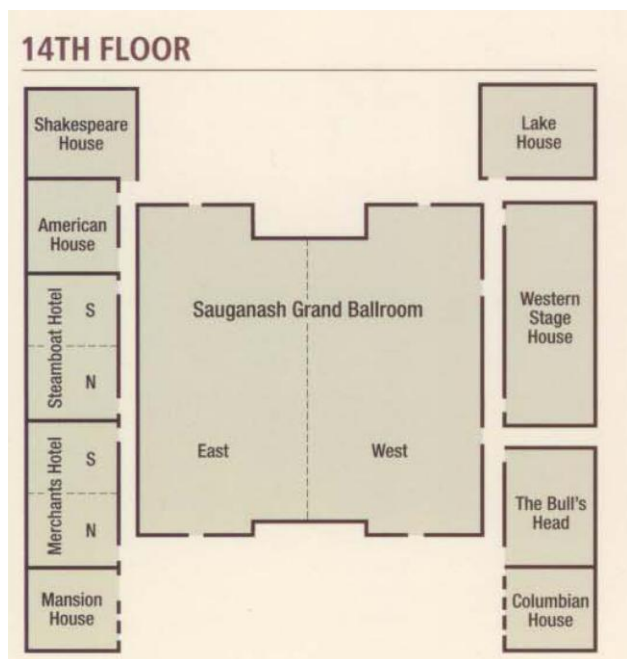


John was instrumental in establishing the National Swine Resource and Research Center. He also was an active participant in establishing similar rodent repositories, the Mutant Mouse Regional Resource Center (MMRRC) and the Rat Resource and Research Center (RRRC) at MU. John was a well-funded and well-respected researcher in the fields of comparative medicine, cryobiology, and reproductive biology. He authored or co-authored over 190 publications. John was a tireless advocate for the development of the Center for Comparative Medicine and his tremendous vision and unique ability to forge fruitful and lasting collaborations among individuals with diverse expertise from all over the world were among his notable strengths. He is missed by all who knew him.

Table of contents

Committees.....	1
Historic Background.....	2
Program.....	3
Speaker and Program Member Profiles.....	7
Abstracts	
Invited Speakers.....	17
Session 1: Building Blocks of Models: Genomics, Bioinformatics, and Metabolism.....	28
Session 2: Clinical Implications: Lesson from Successful Models.....	43
Session 3: War of the Worlds? The Academic--Regulatory – Industry interface.....	57
Session 4: Emerging Models: Regenerative Medicine, Behavior and Bioengineering.....	63
Session 5: Workshop on Developing Transgenic Pig Models.....	70
Index	74

Map of Holiday Inn Chicago Mart Plaza Facilities



*Posters will be available for reviewing in Sauganash West throughout the meeting.

** Vendor exhibits will be in the 14th Floor Foyer.

*** Reception will be held in the Wolf Point Ballroom- 15th Floor

Committees

Organizing Committee

Sherrie Clark, D.V.M., Ph.D.

Assistant Professor of Veterinary Clinical Sciences, University of Illinois

John Critser, Ph.D.

Gilbreath McLorn Professor of Veterinary Pathobiology, University of Missouri

Randall Prather, Ph.D.

Curator's Professor of Reproductive Biology, University of Missouri

Lela Riley, Ph.D.

Professor of Veterinary Pathobiology, University of Missouri

Laurie Rund, Ph.D.

Research Assistant Professor of Animal Sciences, University of Illinois

Lawrence B. Schook, Ph.D. (Chair)

Vice President for Research (Interim), Edward William and Jane Marr Gutgsell Professor, University of Illinois

Scientific Program Committee

Alan L. Archibald FRSE, Ph.D.

Professor and Head of Genetics and Genomics, Chair of Mammalian Molecular Genetics
The Roslin Institute and the Royal (Dick) School of Veterinary Studies, University of
Edinburgh, Scotland, UK

Ina Dobrinski, D.V.M., Ph.D.

Professor and Head, Department of Comparative Biology & Experimental Medicine
College of Veterinary Medicine, University of Calgary, Canada

Merete Fredholm, D.V.M., Ph.D.

Professor, Royal Veterinary and Agricultural University, University of Copenhagen,
Denmark

Jorge Piedrahita, D.V.M., Ph.D.

Professor of Molecular Biomedical Sciences, North Carolina State University

Lawrence B. Schook, Ph.D. (Chair)

Vice President for Research (Interim), Edward William and Jane Marr Gutgsell Professor,
University of Illinois

Graphic Design

Kyle Schachtschneider, University of Illinois

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Swine in Biomedical Research Historical Perspective and Conference Objectives

The **Swine in Biomedical Research Conference 2011** highlights the growing utility of swine models in biomedical research. In the toolbox category, genomic and tissue microarrays and next generation sequencing are just beginning to be appreciated. In the applications category, there is still much to explore beyond the pig's already proven value in immunology, nutrition, transplantation, cardiovascular disease, and orthopedics. New initiatives are warranted in zoonotic diseases, cognitive behavior, bioengineering and regenerative medicine. The pig genome has been completed and sequence information can be used to construct models to develop new means of diagnosing and treating life style-related diseases. The ability to genetically modify and clone pigs further enhances the value of the pig for dissecting disease mechanisms and validating clinical therapies. Recent workshops have focused on utilizing animal sciences expertise traditionally supported by the USDA can support NIH funded investigators that utilizing pig models. These joint USDA and NIH discussions identified cultural differences and the lack of in depth knowledge of needs and opportunities as major rate limiting issues. Hence, this conference has been organized to directly address these acknowledged limitations. First, the Organizing Committee has selected individuals whose research has successfully transcended both health and animal science cultures. Second, the invited speakers will showcase investigations using the pig that demonstrate successful applications as well as promising areas that require engagement across the proposed cultural divide. Finally, we will invite USDA and NIH representations to lead discussions on integrating respective strengths into addressing critical biomedical research issues.

The specific aims are:

Specific Aim 1. *Identify areas of study or methodologies that enhance the utility of pigs as biomedical models.* In particular, the conference will focus on identifying human diseases where traditional rodent models have not proven relevant, where the historical use of swine in this regard may be enhanced, or where no useful models exist today and for which pigs may be considered.

Specific Aim 2. *Identify resource needs and areas in which new approaches or methodologies are required.* One session will be organized by the National Swine Resource and Research Center to showcase approaches proven successful in creating current models as well as present new approaches for creating transgenic pigs.

Specific Aim 3. *Stimulate interactions between researchers working within swine and human disciplines.* To date, the majority of researchers focusing on either porcine physiology and related disciplines are in separate research communities than those conducting clinically focused research. The conference will provide a forum for introductions and to identify joint programs and research targets to further facilitate the utility of the pig.

Specific Aim 4. *Provide an integrated discussion focusing on the regulated use of pigs and regulatory approval pathways associated with the use of pigs for biomedical research.* Barriers for the full utilization of the pig as a biomedical model, e.g. are related to the development of appropriate animal protocols and housing, will be addressed.

Sunday, July 17

- 2:00 - 9:00 Arrivals and Registration (*Mansion*)
- 4:00 - 7:00 Posters Setup (*Sauganash West*)
- 7:00 - 8:30 **WELCOMING OPENING SESSION** (*Sauganash East*)
Session Chairs: Lawrence Schook and Geoff Clark
- 7:00 – 7:30 *Welcoming remarks and summary of 2008 Workshop*
Randall Prather and Lawrence Schook
- 7:30 – 8:00 *Coming of Age: The Pg as a Biomedical Model*
Eiji Kobayashi, Jichi Medical University (SP11)
- 8:00 - 10:00 Reception (Wolf Point Ballroom 15th Floor)
and Review of Posters (*Sauganash West*)

Monday, July 18

- 7:30 - 8:30 **CONTINENTAL BREAKFAST**
- 8:30 - 12:30 **SESSION 1: Building Blocks of Models: Genomics, Bioinformatics, and Metabolomics** (*Sauganash East*)
Session Chairs: Lars Bolund and Martien Groenen
- 8:30 - 9:15 *The Pig Genome Sequencing Project*
Alan Archibald, U. of Edinburgh (SP2)
- 9:15 - 10:00 *Comparative Phenomics: Bioinformatic Tools for Utilizing the Pig*
James Reecy, Iowa State University (SP17)
- 10:00 - 10:30 Coffee Break
- 10:30 - 11:00 *Piglet Model for Pediatric Nutrition and Metabolism*
Jack Odle, North Carolina State University (SP14)
- SELECTED TALKS** from Abstracts Discussion lead by session chairs
- 11:00 - 11:15 *Identification of Genomic Regions Associated with Cryptorchidism in Pigs*
Gary Rohrer, USDA, ARS Clay Center, NE (S1-20)
- 11:15 - 11:30 *Comparison of the Epigenomes of Pig, Mouse and Human Pluripotent Cells*
Sheng Zhong, University of Illinois (S1-27)
- 11:30 - 11:45 *BWS Symptoms in Cloned Piglets are Associated with Hypomethylation at the KCNQ-OT1 CpG Island*
Kevin Wells, University of Missouri (S1-15)
- 11:45 - 12:00 *Shifts in Gut Microbial Community Composition Associated with Degree of Solubility of Dietary Fiber*
Fang Yang, University of Illinois (S1-28)

- 12:00 - 12:15 *DNA Methylation Status and Cloning Efficiency in Swine Nuclear Transfer (NT) donor cells*
Randy Prather, University of Missouri (S1-26)
- 12:15 - 12:30 *Polymorphic Patterns in Toll-like Receptor Genes of Suid Species from Different Geographic Locations*
K. A. Darfour-Oduro, University of Illinois (S1-6)
- 12:30 - 2:00 **LUNCH** (*Sauganash West*)
- 2:00 - 6:00 **SESSION 2: Clinical Implications: Lesson from Successful Models.**
(*Sauganash East*)
Session Chairs: Kate Ackerman and Michael Swindle
- 2:00 - 2:30 *Xeno-Transplantation Models*
Simon Robson, Beth Israel Deaconess Medical Center (SP18)
- 2:30 - 3:00 *Combined SNP Association Study in Human and Pig Related to Subcutaneous Fat Thickness*
Hee-bal Kim, National Institute of Animal Science, Korea (SP12)
- 3:00 - 3:30 *Pig Surgical Models*
Richard Pierson, University of Maryland (SP16)
- 3:30 - 3:45 **Coffee Break**
- 3:45 - 4:15 *Designing Models for Human Cancer*
Chris Counter, Duke University (SP4)
- 4:15 - 4:45 *Pigs in Dental Research*
Susan Herring, University of Washington (SP8)
- 4:45 - 5:00 *Controlled Gene Expression in Multi-transgenic Pigs for Xenotransplantation*
David Ayares, Revivacor (S2-4)
- SELECTED TALKS** from Abstracts Discussion lead by Ning Li
- 5:00 - 5:15 *Development of a Swine Model of Esophageal Pre-Cancer*
L.G. Coghlan, University of Texas M.D. Anderson Cancer Center (S2-7)
- 5:15 - 5:30 *Development of Genetically Modified Pigs Suitable for Diabetes and Its Complications Research*
Hiroshi Nagashima, Meiji University, Japan (S2-26)
- 5:30 - 5:45 *Transgenic Pig Models for Studying Neurodegenerative Diseases*
Xiao-Jiang Li, South China Institute for Stem Cell Biology and Regenerative Medicine (S2-27)
- 5:45 - 6:00 *Elevated Renin and Enhanced Adrenal Steroidogenesis in Ossabaw Swine Model of Metabolic Syndrome*
M. Alloosh, Indiana School of Medicine (S2-1)

- 2:00 - 4:45 **SESSION 3: War of the worlds? The Academic—Regulatory—Industry interface, a world of models, medicines, and scientific frontiers (Merchants)**
Session Chairs: Niels-Christian Ganderup and Nicole Navratil
- 2:00 - 2:30 *War of the worlds? Similarities and Differences of Academic and Pharmaceutical Research*
Peter Glerup, CIT Lab, Lille Skensved, Denmark (SP7)
- 2:30 - 3:00 *From Animal Model to Treatment of Human Disease – Minipigs Supporting Development of New Medicines, Real Life Examples*
Niels-Christian Ganderup, Ellegaard Göttingen Minipigs, Denmark (SP6)
- 3:00 - 3:30 *The Academic-Corporate Juncture – Providing Scientific Evidence for an Investigational New Drug (IND) Application*
Karl H. Schuleri, Johns Hopkins University (SP20)
- 3:30 - 3:45 **Coffee Break**
- 3:45 - 4:15 *The Animal What? The Animal Rule: Developing a New Medicine in Minipigs Without Studies in Patients - the Ultimate Test for any Animal Model*
Stanley Hulet, US Army ECBC/Research and Technology (SP9)
- 4:15 - 4:45 *R&D on an Academic Basis: Production of Genetically Designed Pigs in Denmark and China*
Lars Bolund, Beijing Genomics Institute (SP3)

Tuesday, July 19

- 7:30 - 8:30 **CONTINENTAL BREAKFAST**
- 8:30 - 12:00 **SESSION 4: Emerging Models: Regenerative Medicine, Behavior and Bioengineering (Sauganash East)**
Session Chairs: Amy Wagoner-Johnson and Sherrie Clark-Deener
- 8:30 - 9:00 *Pigs and Regenerative Medicine*
Steve Stice, University of Georgia (SP21)
- 9:00 - 9:30 *Pigs and Bioengineering*
Matthew Wheeler, University of Illinois (S23)
- 9:30 - 10:00 *Social Rank and Primate Health: A Potential Future for *Sus scrofa* Pig Models*
James Anthony, Michigan State University (SP1)
- 10:00 - 10:30 **Coffee Break**
- 10:30 - 11:00 *Pigs as a Model for Infant Development of Cognitive Behavior*
Rod Johnson, University of Illinois (SP10)
- 11:00 - 12:00 **SELECTED TALKS** from Abstracts Discussion lead by Session Chairs
- 11:00 - 11:15 *Characterization of Porcine Skin as a Model for Human Skin Studies Using FT-IR Spectroscopic Imaging*
Rong Kong, University of Illinois (S4-6)

- 11:15 - 11:30 *Bioengineering Hemostasis Using Recombinant Human Fibrinogen Sealants in Swine Surgical Models*
W.H. Velander, University of Nebraska-Lincoln (S4-11)
- 11:30 - 11:45 *Ultrasound Strain Imaging of Arterial Wall Elastic Parameters in a Swine Model of Atherosclerosis*
C.G. Krueger, University of Wisconsin (S4-7)
- 11:45 - 12:00 *A Challenge to Developing Humanized Kidney using Porcine Renal Anlagen as Scaffold*
H. Matsunari, Meiji University, Japan (S4-8)
- 12:00 - 1:00 **WORKING LUNCH** (*Sauganash West*)
- NIFA-NIH program announcement is titled "Dual Purpose with Dual Benefit: Research in Biomedicine and Agriculture Using Agriculturally Important Domestic Species"*
Mark Mirando, National Program Leader for USDA's, National Institute of Food and Agriculture (SP13)
- 1:00 - 5:00 **SESSION 5: NSRRC Workshop on Developing Transgenic Pig Models**
Moderators: *Lela Riley and Randy Prather, NSRRC, U. of Missouri*
- 1:00 - 1:30 *Strategies for Making Genetic Modifications*
Jorge Piedrahita, North Carolina St. U. (SP15)
- 1:30 - 2:00 *Modification of the Male Germline*
Ina Dobrinski, Head, University of Calgary (SP5)
- 2:00 - 2:30 *Zinc-finger Nucleases: New Innovations in Custom-Designed Modification of the Swine Genome*
Jeff Whyte, University of Missouri (SP24)
- 2:30 - 3:00 **Coffee Break**
- 3:00 - 3:30 *Construction of Gene Targeting Vectors for Use in Porcine Fetal Fibroblasts*
Kevin Wells, University of Missouri (S22)
- 3:30 - 4:00 *Regulations and Regulatory Considerations*
Larisa Rudenko, FDA (SP19)
- 4:00 - 5:00 *Discussion and Q&A on Developing Transgenic and Cloned Models*
Lela Riley and Randy Prather, University of Missouri
- Adjourn



Dr. Kate Ackerman is a physician scientist and Assistant Professor in the departments of Pediatrics and Biomedical Genetics at the University of Rochester Medical Center in Rochester, NY. Her laboratory focuses on understanding mechanisms of development of specific structural birth defects that cause critical illness in the human neonate. Dr. Ackerman's group focuses mainly on birth defects of the diaphragm and uses a variety of approaches including forward genetic screening to identify genetic contribution to disease in mice, humans, and swine. Dr. Ackerman's work is currently funded by the NIH. Clinically, Dr. Ackerman is board certified in Pediatrics and Pediatric Critical Care and practices medicine in the Division of Pediatric Critical Care Medicine at Golisano Children's Hospital at Strong.



James C. (Jim) Anthony, Ph.D. earned his bachelor's degree from Carleton College in 1971, his M.Sc. from the University of Minnesota in 1975, his Ph.D. from the University of Minnesota in 1977, and a postdoctoral fellowship award at Johns Hopkins University School of Hygiene and Public Health (1977-78). Thereafter, he was appointed to the JHU faculty, where he used clinical and epidemiological research protocols to study the occurrence, causes, and prevention of neuropsychiatric disturbances with a special focus on hazards of psychoactive drug use, including drug dependence. In 2003, he left his professorship at Johns Hopkins to return to the midwest and a professorship at Michigan State University, the current base of his research, which includes, with NIH support, development of an international network of researchers who are using a pig model in basic and applied research on various aspects of neuroscience, behavioral, and social science research that is pertinent to human neuropsychiatric and behavioral disturbances. He is an elected Fellow in the American College of Neuropsychopharmacology, the College on Problems of Drug Dependence, and the American Psychopathological Association.



Professor Alan L. Archibald is Head of the Division of Genetics and Genomics at The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh. Professor Alan Archibald is internationally recognised in the field of farm animal genetics and genomics research. He co-led the first international farm animal genome project – PiGMaP. Together with Professor Chris Haley he was awarded the Royal Agricultural Society of England's Research Medal in 1997 for research work of benefit to agriculture. He is a member of the Steering Committee of the Swine Genome Sequencing Consortium under whose auspices the pig genome is being sequenced. His research is concerned with understanding the genetic control of complex traits, including production efficiency, product quality and host response to infectious disease, mainly in pigs and cattle. He is a Fellow of the Royal Society of Edinburgh which is Scotland's National Academy of Science and Letters.



Lars Bolund, M.D., DMSc., is Professor of Clinical Genetics at Aarhus University, Denmark, and Adjunct Professor of Human Genetics at Copenhagen University, Denmark, as well as at HuaDa JiYin (BGI) Shenzhen, China. He got his medical and scientific degrees from the Karolinska Institutet in Stockholm, Sweden, and has held many different research appointments in Sweden, the United Kingdom, the United States, Denmark and China. He now spends about half his time in Shenzhen, China, and the rest in Denmark. He has served on numerous national and international boards and committees and received many honors, including The August Krogh Prize, Knighthood of the 1st Class of the Royal Danish Order of Dannebrog and the Chinese National Friendship Award. He has published more than 300 scientific articles, separate database submissions and patents (scientific abstracts excluded) in the fields of genome/gene structure and function in cell biology and clinical genetics, molecular cell pathology of complex diseases, development of genetically designed animal models of complex disease processes and somatic gene therapy.



Geoffrey J. Clark, Ph.D., received his B.S. in Biochemistry and Molecular Biology and his Ph.D. in Molecular Oncology from the University of Manchester, United Kingdom. He is currently an Associate Professor in the Department of Medicine at the J.G. Brown Cancer Center at the University of Louisville. After completing Postdoctoral Fellowships at the University of Southern California, Los Angeles and the La Jolla Cancer Research Foundation, Dr. Clark worked as a Research Associate and Research Assistant Professor at the University of North Carolina at Chapel Hill. Currently, Dr. Clark serves as an Editorial Board Member for the *Biochemical Journal*. He is currently leading research projects concerned with tumor suppression in kidney, lung cancer and a porcine model of breast cancer.



Sherrie G. Clark, DVM, PhD, Dipl. ACT attended VPI&SU and received her B.S. in Animal Sciences with minors in Biology and Chemistry in 1992. She continued her education by attending the VA-MD Regional College of Veterinary Medicine and graduating in 1996. Upon graduation, she was accepted in to a dual residency/M.S. degree program in Theriogenology in the Department of Veterinary Clinical Medicine at the University of Illinois. During her residency, she completed the Executive Veterinary Program in Swine Health Management. She received her M.S. in 1999 under the supervision of Dr. Gary C. Althouse. Dr. Clark subsequently remained at the University of Illinois to pursue a Doctorate of Philosophy in the Department of Animal Sciences under the supervision of Dr. Matthew B. Wheeler. In 2003, she attained her PhD in Reproductive Physiology and passed the board certification examination in Theriogenology. That same year, she has accepted a tenure-track assistant professor position in the Department of

Veterinary Clinical Medicine Farm Animal Reproduction, Medicine and Surgery section at the University of Illinois where she remains today.



Christopher M. Counter, Ph.D., is an Associate Professor in the Department of Pharmacology & Cancer Biology at the Duke University Medical Center and serves as co-leader of the Cancer Biology & Regulation Program in the Duke Cancer Institute. His research focuses on key characteristics of cancer cells, in particular cell immortalization and transformation. His studies on cell immortalization led to the discovery that telomere shortening is overcome by activation of the enzyme telomerase during tumorigenesis. His studies on transformation revealed the importance of the Ral and eNOS pathways in Ras-oncogenic signaling. He was the recipient of the Kimmel Foundation Scholar Award, Leukemia and Lymphoma

Society Scholar Award, and the John J. Abel Award in Pharmacology from ASPET and is currently a charter member of the NIH Cancer Molecular Pathobiology Study Section and serves on the Scientific Advisory Board for the Colorado Cancer Center.



Ina Dobrinski, Dr. med. vet., M.V.Sc., Ph.D., Dipl. ACT., is Professor of Reproductive Biology and Department Head in Comparative Biology & Experimental Medicine in the Faculty of Veterinary Medicine at the University of Calgary. She joined the University of Calgary in 2008 after 11 years at the University of Pennsylvania, School of Veterinary Medicine, where she was a Professor of Reproduction, the Director of the Center for Animal Transgenesis and Germ Cell Research, and the Marion Dilley and Robert George Jones Chair in Reproduction. Dr. Dobrinski's group studies the biology of male germ line stem cells in non-rodent models, including primate, porcine and small ruminant models. Dr. Dobrinski has strong interests in using germ line modification as an approach to generating transgenic non-rodent animal models for biomedical research, and in harnessing the plasticity of germ line stem cells to acquire pluripotency and to differentiate

into different cell types for tissue regeneration.



Merete Fredholm, Ph.D., is a Professor of Animal Genetics in the Department of Basic Animal and Veterinary Sciences, The Royal Veterinary and Agricultural University (RVAU) in Copenhagen, Denmark. She received her D.V.M. degree in 1982, a Ph.D. in Animal Genetics in 1986 and a Doctorate of Veterinary Science in 1996, all from RVAU. Her Ph.D. thesis was titled "Immunogenetic investigations of the major histocompatibility complex in Danish Swine Breeds" and her doctoral thesis was titled "A molecular approach to investigation of basic mammalian genetics". Dr. Fredholm's current research interests are directed towards gene mapping and genome analysis in farm animals with emphasis on host pathogen interaction, disease genetics and comparative genomics. Furthermore, she takes part in the international efforts towards sequencing the swine genome. She has published 65 publications in international, peer reviewed journals.



Niels-Christian Ganderup, M.Sc. Biology, trained first in experimental biology and has had special experience of toxicology and ecotoxicology. Since 2005 he has been generally concerned with best use of the minipig in the biomedical sciences. His principal role has been active engagement world-wide with academic, pharmaceutical and other industrial research organisations and CROs in comparing the value of the minipig and other species in research and testing programmes in pharmacology and toxicology. His broad interests cover the 'Can I ...?', 'How can I ...?' and 'Who can show me how to ...?' questions. This has led him to facilitate cross-company and inter-organisation research consortia to extend scientific knowledge about the minipig and its uses in biomedical research. To further increase collaboration and to improve knowledge of techniques Niels-Christian Ganderup founded the Minipig Research

Forum, a non-profit organisation, which organises annual scientific conferences in Europe and North America about all aspects of the use of minipigs in biomedical research. He is a regular speaker at conferences and is often consulted by universities, CROs and industrial groups about many aspects of the use of the minipig ranging from health and husbandry to training in experimental techniques and the development of new procedures.



Peter Glerup, DVM, MSc obtained his veterinary degree from the University of Copenhagen, with laboratory animal research and toxicology as areas of specialization. He has also received specialist training in general toxicology and surgery, and has obtained a postgraduate diploma in toxicology from the University of Copenhagen, and a postgraduate diploma from the University of Copenhagen in the science of radioactive isotopes. In addition, Peter has a master degree in laboratory animal science from the University of Copenhagen and the University of Helsinki. Peter joined LAB Research (Scantox) in 1996 and has extensive experience within preclinical pharmacology and toxicology testing. In 1999 he was appointed Chief Veterinary Surgeon and in 2005 as Director of Toxicology Sciences. Peter has been

working with landrace pigs and minipigs for more than 15 years and has extensive scientific and practical experience in numerous study types and procedures with pigs and minipigs.



Martien Groenen, Ph.D., (1983, Leiden University, The Netherlands) has been a professor in Animal Genomics at Wageningen University since 2002. He has been involved in mapping the genomes of various farm animals since the beginning of the 1990s. In 2004, this resulted in three Nature publications describing the genome sequence and SNP variation of the chicken. He plays a prominent role in the international chicken, great tit and swine genome sequencing committees. In 2009 he was awarded a prestigious advanced ERC research grant for his research project 'Molecular characterization of genetic factors in the pig under selection during speciation, domestication and breeding'. He is member of the Editorial boards of

numerous scientific journals and he is leading or co-author on more than 180 papers in peer-reviewed journals. In 2011 he became a faculty member of the Netherlands Bioinformatics Centre, NBIC. Currently his main research activities at Wageningen University are focused on the detailed molecular characterization of the genome of the pig and other Suids.



Susan W. Herring, Ph.D., was trained at the University of Chicago (B.S. in Zoology and Ph.D. in Anatomy) where she studied the comparative cranial anatomy of pigs and their close relatives. Feeling the need to work with live animals, she moved to the College of Dentistry, University of Illinois at Chicago as an NIH postdoctoral fellow, where she developed techniques to record muscle activity and jaw movement from chewing minipigs. She remained at UIC as a faculty member until 1990, rising to Professor of Oral Anatomy and Anatomy. In 1990 she took up her current position in Seattle as Professor of Orthodontics and Oral Biology at the University of Washington. Her work on the biology of the craniofacial musculoskeletal system of pigs has been continuously funded by NIH for over 30 years. She became a fellow of AAAS in 1992 and received the Craniofacial Biology Research Award from the International Association of Dental Research in 1999. She serves on the editorial boards of several journals and has held office in several societies, including the Society for Integrative and Comparative Biology (chair, Vertebrate Morphology, 1983-4), International Association of Dental Research (president, Craniofacial Biology, 1997-8), International Society of Vertebrate Morphology (president, 1994-7), and AAAS (member-at-large, Section R, 2008-12).



Stanley W. Hulet, Ph.D., DABT, received his B.S. (biology/psychobiology) from Lebanon Valley College and his Ph.D. in Neuroscience from Pennsylvania State University in Hershey, PA. His graduate research focused on metabolism of iron and regulation of iron transport proteins within the brain. He went on to receive a National Research Council Postdoctoral fellowship to perform research at the US Army Medical Research Institute of Chemical Defense. In 2001, he relocated to the US Army Edgewood Chemical and Biological Center where he currently serves as a study director. His research focuses on protecting soldiers from the hazards of chemical weapons. In 2010, Dr. Hulet became a board certified Diplomate of the American Board of Toxicology.

He currently resides in Joppa, MD with his wife and 2 daughters.

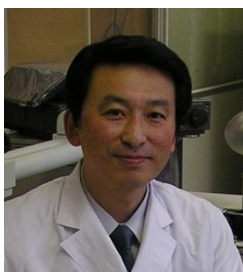


Dr. Rodney Johnson is a professor of integrative immunology and behavior in the University of Illinois Department of Animal Sciences and Director of the Division of Nutritional Sciences. His research investigates neuroinflammation and its effects on brain and cognitive development and aging. A special focus is on how diet influences the communication between the immune system and brain. Johnson earned a B.S. from Truman State University and a M.S. and Ph.D. from the University of Illinois. After post doctorate training at Iowa State University, he joined the U. of I. faculty in 1993. Johnson has published over 100 peer reviewed papers and is a University Scholar.



Heebal Kim, Ph.D., received his M.Sc and B.Sc in Animal Science from the Seoul National University in Korea. He obtained his Ph.D in Animal Genomics (with Marlene Emara) from the University of Delaware (2003) and his M.Sc in Computer and Information Science (with Keith Decker) as a dual major from the University of Delaware (2003). He did his postdoctoral training with Jurg Ott (Statistical Genetics) at the Rockefeller University (2004). He is an associate professor in the Department of Food and Animal Biotechnology at the Seoul National University. His primary research interests include computational molecular evolution, genome-wide association study in complex trait and genome-wide expression profiling. He is especially interested in developing method for genomic selection and identifying genomic signature of

domestication in animals. Currently, he is working as a visiting scholar in Rasmus Nielsen's Lab at the UC Berkeley.



Eiji Kobayashi, M.D., Ph.D has two positions as a visiting professor of Center for Development of Advanced Medical Technology (CDAMTec), Jichi Medical University (JMU), Japan and a chief scientific adviser, Otsuka Pharmaceutical Factory, Inc. He graduated from JMU at 1982 and has been working in Translational Research Center during recent 10 years as both Professor positions of Center for Molecular Medicine (CMM) and Department of Surgery, JMU. He has continuously been a supervisor of more than 200 cases of living-related liver transplantation in the university hospital and

made more than 500 research papers. From 2003, he had been a Director of Center for Experimental Medicine (CEM), JMU and conducted all living animal experiments in this university. At the beginning of starting his lab, he conducted two big research projects of production of engineered rats and biomedical pigs for translational research. Now, he scales up pre-clinical approach using a medical pig system for development of innovative medical treatment for human health and disease. He succeeded in developing clone pigs and is focusing on an “*in vivo bioreactor*”, where human stem cells can transdifferentiate in their organogenesis. Using this technology, he would like to make the transplantable organ without donors.



Ning Li, Ph.D., was co-educated in Dublin University of Ireland and Beijing Agricultural University of China and titled with Ph D in 1991. From 1991 to 1994 he spent three years in German, Japan and USA as a post doctor or visiting scholar. He was appointed as Director of State Key Laboratory for Agrobiotechnology in 2005, and elected to academician of the Chinese Academia of Engineering in 2007. Currently he is member of the Editor Board of Animal Biotechnology and AJAS, and member of the International Committee of World Congress of Genetics Applied to Livestock Production. Prof Li is “Changjiang Honored Professorship”, and has been crowned National Award for Scientific and Technology in 2000, 2001, 2003, 2005, 2009 and 2010. He is also awarded many other honored titles such as

Major Contributors to State High-Tech Research and Development Program. Prof Li has been the PI for 36 projects during last decade, including State Key Projects of “863” program and “973” program as well as PigBioDiv II project funded by the EU Five Frame Program. Prof. Li’s work is mainly focused on identification of functional genes influencing animal production traits such as growth, meat quality and genetic resistance. He is also leading a team working on animal cloning and transgenics for improvement of production traits of farm animals as well as generation of swine model for human diseases. So far as the first author or correspondent, Prof. Li has published two books and more than 280 international peer-reviewed papers in Nature, PNAS, Genome Research, Plos Genetics, Nuclear Acid Research, Plos ONE, Journal of Immunology, BOR, Journal of Virology, etc..



Mark Mirando, Ph.D., is National Program Leader of Animal Nutrition, Growth and Reproduction with the USDA’s National Institute of Food and Agriculture (NIFA) where he has provided leadership for competitive grant programs in animal reproduction and animal growth for the past ten years. More recently, he has also provided leadership for competitive grant programs in animal genomics, biotechnology risk assessment, and an interagency program with NIH. He is currently completing his fifth term as Editor for the Journal of Animal Science and has served on the editorial boards for 3 journals. Before joining NIFA, Dr. Mirando served on the faculty of the Department of Animal Sciences at Washington State University from 1990 to 2000. During his tenure at Washington State University, he was actively engaged in a variety of research, teaching, advising and

service activities. His research on cell signaling in the uterus of domestic ungulates attained international recognition and was supported by grants from the USDA National Research Initiative and the National Institutes of Health. In addition to instructing the largest undergraduate course in his department for 10 years, Dr. Mirando taught graduate-level courses in physiology and mentored 11 graduate students and three postdoctoral associates. His own postdoctoral studies in uterine biology were performed at the University of Florida after completing his PhD in 1987 and MS in 1982 at the University of Connecticut in the physiology, endocrinology and biochemistry of reproduction.



Nicole Navratil has been working with Marshall BioResources and involved with the Gottingen Minipigs for 4 years. She regularly participates in hands on training sessions for facilities new to working with minipigs, and she has been involved in several projects looking at enhancing minipig welfare. She is currently the Scientific Sales Representative at Marshall BioResources and serves as a resource for facilities working with minipigs. She is also a member of the Minipig Research Forum, a non-profit organization which provides resources on the use of minipigs in biomedical research and holds annual scientific conferences in both Europe and North America. She has been very active in organizing the Minipig Research Forum meetings held in North America. She received her Masters of Science in Biomedical

Anthropology from the University of Binghamton.



Jack Odle, Ph.D., received his B.S. degree with highest honors in Animal Science from Purdue University in 1982. Graduate degrees (M.S. and Ph.D.) were completed in 1989 from the University of Wisconsin-Madison, focused in Nutritional Biochemistry. After five years as Assistant Professor of Animal Science at the University of Illinois, he was recruited to the Department of Animal Science at North Carolina State University in 1995 and named William Neal Reynolds Distinguished Professor in 2005. He is a member of the Genomics and Biotechnology Faculties, the Center for Gastrointestinal Biology and Disease, and the Center for Comparative Medicine and Translational Research at NCSU. Dr. Odle's research program is focused on the "*Developmental Biochemistry of the Neonate*." His research has relevance to both production agriculture and to medical science in that his laboratory utilizes newborn piglets as a model to study metabolism throughout postnatal development. His research, focused on lipid metabolism and on intestinal growth and development, has received multiple national awards from both animal and nutrition research societies including dual *young investigator awards* in 1995, the *AFIA Nonruminant Nutrition Award* in 2004 and the *Animal Growth and Development Award* in 2010. He serves routinely on USDA grant review panels and has served on the editorial boards of the *Journal of Animal Science* and the *Journal of Nutrition*, including six years as associate editor. He presently serves as associate editor of *Advances in Nutrition* and for the *Journal of Animal Science and Biotechnology* and Director of a USDA transdisciplinary training grant focused on functional foods, bioactive food components and human health.



Jorge Piedrahita, Ph.D., obtained his MSC in Reproductive Physiology and his PHD in Cell and Developmental Biology from the University of California, Davis under the supervision of Dr. Gary Anderson. He then moved to the University of North Carolina, Chapel Hill where he completed his Postdoctorate training in stem cells and homologous recombination with Dr. Oliver Smithies and Dr. Nobuyo Maeda. He started his academic career at Texas A&M University where he rose from Assistant Professor to Full Professor. Dr. Piedrahita is presently at the College of Veterinary Medicine, North Carolina State University where he is a professor in the department of Molecular Biomedical Sciences. Additionally, he is the interim Director for the Center for Comparative Medicine and Translational Research. Dr. Piedrahita has received numerous research awards during his career including the Basil O'Connor Fellowship from the March of Dimes, Pfizer research award, Litwack award and Huffman Leadership award. Jorge Piedrahita's laboratory is primarily interested in the understanding of the role of imprinted genes in embryo development and in disease, and the development of transgenic animals of use in human and veterinary medicine, and in agriculture. Towards this end, we combine techniques in functional genomics, cell biology, embryo manipulation, and molecular biology. Specifically, our efforts concentrate in the use of gene expression profiles in models of imprinting dysregulation and in functional genomic analysis of candidate genes. Presently, our research is focused on: a) The identification and functional analysis of imprinted genes in humans and swine. b) The characterization of embryonic stem cell lines and induced pluripotent stem cells from a variety of mammals. c) The development of porcine models of human disease using somatic cell nuclear transfer with transgenic somatic cells.



Richard N. Pierson III, MD, FACS, is Professor of Surgery at the University of Maryland, and Chief of Surgery at the Baltimore Veterans Administration Medical Center. He received his training from Columbia University, the University of Michigan, Massachusetts General Hospital, and Papworth Hospital in Cambridge, England. His main clinical interest is in heart and lung transplantation. He is the immediate past President of the International Xenotransplantation Association (IXA). He is a member of a variety of professional organizations, and has authored over 120 scientific articles, mainly dealing with transplantation and mechanical circulatory support.



Randall S. Prather, Ph.D., Since 1982 Dr. Prather's research has focused on the early mammalian embryo. He earned his BS and MS from Kansas State University, and PhD and Postdoc from the University of Wisconsin-Madison. While at Wisconsin he cloned some of the first cattle by nuclear transfer and the first pigs. His group at the University of Missouri has created miniature pigs that have the alpha 1,3 galactosyltransferase gene knocked out, thus paving the way for xenotransplantation; and have developed pigs that have cystic fibrosis, thus providing the first whole animal model that can be used to study the disease. His lab has made over 475 cloned pigs at MU representing over 25 different genetic modifications for agriculture and medicine. He is a co-Director of the NIH-funded National Swine Resource and Research Center. In addition to his transgenic pig research, he and his collaborators have identified newly described genes

in the reproductive tissues of pigs and cattle that will help develop an understanding of the pattern of gene expression to reduce the 30% loss of pregnancies that occurs in mammals. He has helped to secure over \$81.2 M in research funding, and has over 240 peer-reviewed publications and 103,000 GeneBank submissions. He is currently a Curators' Professor with the title Distinguished Professor of Reproductive Biotechnology in the Division of Animal Science at the University of Missouri.



James M. Reecy, Ph.D., received his B.S. (Animal Science) from South Dakota State University, an M.S. (Animal Science) from the University of Missouri-Columbia, and Ph.D. (Animal Science) from Purdue University. He performed postdoctoral training in the Cell Biology department at Baylor College of Medicine. His research has focused on the elucidation of genetic mechanisms underlying nutrient composition of meat, resistance to bovine respiratory disease and pinkeye infection. In addition, his group is actively developing bioinformatic tools and resources to facilitate genomics research in livestock species. He currently serves as the lead Coordinator of the NRSP-8 Bioinformatics project (www.animalgenome.org). In addition, he is the Director of the Office of Biotechnology at Iowa State University, which oversees 12 core research facilities, which serve researchers in five colleges. Furthermore, the Office of Biotechnology provides resources and training to enhance elementary, middle school

and high school biotechnology education. Dr. Reecy is also a section editor for BMC Genetics. He has active collaborations on the sequencing of the bison and water buffalo genomes, epigenetic reprogramming of fetal tissue, and reproductive traits in cattle.



Lela Riley, Ph.D., is a Professor in the Research Animal Diagnostic Laboratory (RADIL) at the University of Missouri. Dr. Riley is the Principal Investigator for the National Institutes of Health (NIH)-funded National Swine Resource and Research Center and one of the four NIH-funded Mutant Mouse Research and Resource Centers nationwide. She is also a Co-Investigator on the NIH-funded National Rat Resource and Research Center. These Centers develop and characterize new animal models, acquire valuable genetically-modified models from other investigators worldwide and serve as a resource for investigators in all fields of medicine. Dr. Riley's laboratory is involved in the development of animal models of human diseases, the study of infectious agents found in laboratory animals, and the development of novel diagnostic assays. A major emphasis of her research has been the isolation and characterization of emerging

pathogens and development of methods to detect these agents.



Dr. Simon C. Robson, MD, Ph.D., is a physician scientist in the Liver Center and Transplant Institute at the Beth Israel Deaconess Medical Center, Harvard University in Boston. He was appointed as Professor in Medicine at the Harvard Medical School in 2006. His area of basic science research involves the CD39 related ectonucleotidases, vascular and immune cell expressed ectoenzymes that hydrolyze extracellular nucleotides to adenosine and derivatives. CD39 family members are crucial in modulating vascular inflammation and immune responses in transplanted organs, as well as in the liver and gastrointestinal tract. His laboratory, in collaboration with the TBRC, MGH in Boston MA, St. Vincent's Hospital, Melbourne, Australia and the NSRRC, Columbia MO addresses alterations in coagulation in solid organ and islet xeno and allo transplantation by small and large animal genetic manipulations.



Larisa Rudenko, Ph.D., is Senior Advisor for Biotechnology and Director of the Animal Biotechnology Interdisciplinary Group at the Center for Veterinary Medicine (CVM), FDA. In this capacity, she has worked within the agency, at the U.S. Government (USG) coordinating level, and internationally with public and private sector stakeholders to develop a coherent, transparent, science-based policy for the regulation of animal biotechnology. Dr. Rudenko serves as an expert on Food and Agriculture Organization (FAO) – World Health Organization (WHO) Codex Alimentarius Task Forces, FAO-WHO Expert Consultations, Organization for Economic Cooperation and Development (OECD) Working Groups, international and various other review committees and scientific advisory panels. Prior to her FDA appointment, Dr. Rudenko held ownership and senior management positions in several policy, technical, and regulatory support consulting firms. Dr. Rudenko received her AB from Bowdoin College and her PhD in Cellular and Molecular Pharmacology from the State University of New York at Stony Brook, following dissertation research at the Brookhaven National Laboratory. She is also a Diplomate of the American Board of Toxicology.



Laurie Rund, Ph.D., is a Research Assistant Professor in the Department of Animal Sciences at the University of Illinois. She received her B.S. from the University of Illinois, a M.S. from Texas A&M University and completed her doctoral research in reproductive physiology at The University of Georgia. Following postdoctoral work at The Jackson Laboratory where she worked on a transgenic mouse model of cerebellar developmental, Laurie returned to the University of Illinois. Presently she is leading an NIH-sponsored project focused on the development of a pig model of breast cancer.



Lawrence Schook, Ph.D., Vice President for Research for the University of Illinois, received his B.A. from Albion College and his M.S. (microbiology) and Ph.D. (microbiology and immunology) from Wayne State School of Medicine. After postdoctoral training at the Institute for Clinical Immunology, Berne Switzerland and at the University of Michigan, he has held positions at the Medical College of Virginia, University of Minnesota, and the University of Lausanne. He is a Fellow of the American Association for the Advancement of Science. His research has focused on genetic resistance to diseases and in the development of the pig as a model for biomedical research. Dr. Schook is the founding editor of *Animal Biotechnology* and was the Director of the Food Animal Biotechnology Center and Associated Dean for Research at the College of Veterinary Medicine and U. Minnesota. During that tenure he chaired the AAVMC Research Deans and Directors Committee. Currently at the U. Illinois, he is a Theme Leader in the Institute for Genomic Biology, his laboratory has developed human-pig comparative radiation hybrid map and characterized the swine MHC. Dr. Schook has been recognized as a University Scholar, a Fellow at the National Center for Supercomputer Applications and is the recipient of the Pfizer Animal Health Award. He currently serves as the Co-Chair, International Swine Sequencing Consortium and Chairs of the Steering Committee of the Alliance for Animal Genome Research. He has active collaborations for development of clinical models and most recently his laboratory has focused on technology platforms for using DNA sequence information to target genetic manipulations.



Dr. Karl H. Schuleri, MD received his medical degree from the Humboldt University of Berlin, Germany, and was trained in cardiovascular surgery. He completed a Cardiovascular Research Fellowship at the Weill Medical College of Cornell University, New York, NY and at the Johns Hopkins University, Baltimore, MD. He is currently appointed as a staff researcher in the Division of Cardiology at Johns Hopkins University. His research interests include CT and MRI based assessment of myocardial pathophysiology, image guided therapy approaches and imaging based assessment of cardiac cell therapy. Dr. Schuleri's research projects in recent years were focused on preclinical testing of novel cell therapies in porcine models.



Steven L. Stice is a professor and director of the University of Georgia Regenerative Bioscience Center. He is a Georgia Research Alliance Eminent Scholar endowed chair and professor of animal science in the UGA College of Agricultural and Environmental Sciences. Throughout his career he has published and lectured internationally on animal cloning and stem cells as reported on in the NY Times, USA Today, Time Magazine, CBS, NBC and ABC News broadcasts. In 2001, three of the human embryonic stem cell lines that Stice and BresaGen Inc. derived were approved for federal funding by President Bush. He was named one of the 100 Most Influential Georgians by *Georgia Trend* magazine. Prior to joining the University of Georgia, Stice was a co-founder and chief scientific officer at Advanced Cell Technology, Inc., a stem cell company that is currently in clinical trials for a rare disease affecting sight. His current research is to understand the mechanisms involved in human and animal adult and embryonic stem cell differentiation.



M. Michael Swindle, D.V.M. is Professor and Chairman of the Department of Comparative Medicine at the Medical University of South Carolina. He also holds a Professorship in the Department of Surgery. Dr. Swindle received his B.S. degree and his D.V.M. degree, both from Texas A & M University and is a Diplomate of the American College of Laboratory Animal Medicine and the European College of Laboratory Animal Medicine. He was in the U.S. Army Veterinary Corps from 1969 to 1972 and following a stint in private veterinary practice he was appointed to the faculty of Johns Hopkins Medical School from 1979-1985. At Johns Hopkins he directed the surgical research laboratories. He has many books, publications and oral presentations dealing with porcine surgical models and has received research awards from five professional associations in recognition of his work. He currently serves on scientific committees in both the US and EU. Recently he was appointed as an Honorary Professor at Aarhus University in Denmark and was inducted as an Outstanding Alumnus of Texas A&M University.



Professor A. J. Wagoner Johnson received her BS in Materials Science and Engineering from The Ohio State University in 1996, and MS and PhD in Engineering from Brown University in 1998 and 2002, respectively. She joined the Department of Mechanical Science and Engineering at the University of Illinois first as Research Faculty and then as an Assistant Professor in May of 2005. Her research interests are in fabrication and characterization of biomaterials for bone repair and replacement, cell and tissue mechanics, and tissue engineering. She received the Alice L. Jee Memorial Award at the Sun Valley Workshop on Skeletal Tissue Biology in August 2008, an Honorable Mention for the 2009 Early Career Faculty Fellow Award from The Minerals, Metals, and Materials Society, and the Engineering Council Award for Excellence in Advising in 2009, and the Arnold O. Beckman Award from the University of Illinois in 2011. Dr. Wagoner Johnson is a part-time faculty member of the Beckman Institute for Advanced Science and Technology; has affiliations with the Institute for Genomic Biology, the Department of Bioengineering, the Computational Science and Engineering Program at the University of Illinois; and is a Visiting Professor in the Mechanical Engineering and Materials Science department at Washington University in St. Louis.



Kevin D. Wells is currently an assistant professor at the University of Missouri in the Animal Sciences Division. His lab is primarily concerned with application of genetic engineering to animal agriculture. A portion of his time is also obligated to the National Swine Research Resource Center, which has a goal of providing pig models for the NIH research community. Wells completed his PhD at North Carolina State University in a swine genetic engineering lab. During his post doc at USDA Beltsville he worked on control of transgene expression. Wells then obtained a Research Geneticist position at USDA where he worked on genetic engineering in pigs and cattle. In 2001 Wells joined PPL Therapeutics as a Senior Scientist to head a project to produce human antibodies in genetically modified pigs. After the U.S. division of PPL Therapeutics spun out to become Revivicor, Inc., Wells became Head of the Department of Embryology. In 2007, Wells returned to academia to start a new lab at the University of Missouri. His current lab is primarily concerned with genetic engineering in livestock.



Matthew B. Wheeler, Professor at the University of Illinois at Urbana-Champaign, has a doctoral degree from Colorado State University in physiology and biophysics. He has conducted more than 25 years of research on embryo/developmental biology, in vitro embryo production, stem cells, cloning, transgenic livestock and regenerative biology. Dr. Wheeler has developed and characterized genetically modified swine with increased milk production and weaning weights. He has also been examining how mesenchymal stem cells can be used clinically in bone and soft tissue engineering strategies. Dr. Wheeler has been a frequent invited speaker at national and international scientific meetings. He recently served as the President of the International Embryo Transfer Society and now is the Society's Treasurer. Dr. Wheeler has been recognized as a University Scholar and as a Life-Time Member Brazilian Embryo Transfer Society. Dr. Wheeler's responsibilities at the University of Illinois include teaching and research in biotechnology and reproductive biology in the Departments of Animal Sciences, Veterinary Clinical Medicine and Bioengineering. He is also a member of the Beckman Institute for Advanced Science and Technology and the Institute for Genomic Biology.



Dr. Jeffrey Whyte received his Ph.D. (cellular and molecular toxicology) from the University of Waterloo, Ontario, Canada. He performed postdoctoral training in Missouri at the Columbia Environmental Research Center, USGS Biological Resources Division. He joined the Department of Biomedical Sciences at the University of Missouri in 2006, working with Dr. Randall Prather and Dr. Harold Laughlin to develop transgenic pig models for cardiovascular research. His current research focuses on innovative methods to produce genetically modified pigs as models for biomedicine and to study the role of epigenetic regulation during early development in cloned swine. Techniques being explored by Dr. Whyte include DNA modification with zinc finger nucleases and porcine genomic analysis via high throughput sequencing. Dr. Whyte is currently a Research Assistant Professor in the Department of Animal Sciences at the University of Missouri. He is a co-investigator in a number of collaborative research efforts, including analysis of DNA methylation in pig embryos (Utah State University), transgenic swine as a model for juvenile diabetes (University of British Columbia), and porcine gene modification with zinc finger nucleases (Sigma-Aldrich).

Invited Speakers

SP1.

Social rank and primate health: a potential future for *Sus scrofa* pig models

James C. Anthony

Michigan State University

We are developing an international network to foster new collaborative research at the intersections of biobehavioral and social research on human and non-human primates with biobehavioral and social research in the *Sus scrofa* and other pig models. In this presentation, I will outline a background of primate and swine research that motivates this line of studies. The intent is to extend past research on pig responses to ethanol and opioids, as well as other psychoactive compounds. I will stress opportunities for collaborative research on monozygotic twin piglets as well as social housing experiments that may accelerate current lines of research on social rank and health that might prove to have a clinical translational character.

SP2.

The pig genome sequencing project

A. L. Archibald¹ on behalf of the Swine Genome Sequencing Consortium²

¹ The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, United Kingdom; ² (<http://piggenome.org>)

The pig genome is being sequenced and characterised under the auspices of the Swine Genome Sequencing Consortium. The sequencing strategy followed a hybrid approach combining hierarchical shotgun sequencing of BAC clones and whole genome shotgun sequencing. Assemblies of the BAC clone derived genome sequence have been annotated using the Pre-Ensembl and Ensembl automated pipelines and made accessible through the Pre-Ensembl / Ensembl browsers. The current annotated genome assembly (Sscrofa9) was released with Ensembl 56 in September 2009. A revised assembly (Sscrofa10) is under construction and incorporates whole genome shotgun sequence (WGS) data providing >30x genome coverage. The WGS sequence, most of which comprise short Illumina/Solexa reads, were generated from DNA from the same single Duroc sow as the source of the BAC library from which clones were preferentially selected for sequencing. In accordance with the Bermuda and Fort Lauderdale agreements and the more recent Toronto Statement the data have been released into public sequence repositories (Genbank/EMBL, NCBI/Ensembl trace repositories) in a timely manner and in advance of publication. Analysis and annotation of this draft pig genome sequence is under way. Progress with this characterisation of the pig genome will be reported and the value of the sequence and associated tools to biomedical research community presented.

SP3.

R&D on an academic basis: production of genetically designed pigs in Denmark and China

Lars Bolund

Institute of Human Genetics, University of Aarhus, Denmark, and HuaDa JiYin/BGI, Shenzhen, China.

Some ten years ago we established a Sino-Danish consortium which has been supported by the Danish Advanced Technology Foundation (HTF), Chinese HuaDa JiYin/BGI and other funding agencies to develop the pig as a production animal and disease model. We made a pilot survey of the pig genome and have later participated in the International Swine Genome Sequencing Consortium. Based on the completed genome sequence of the pig genome, genotype-assisted breeding to improve production pig health has been initiated. Pig models for dysregulatory/degenerative human diseases have been created with an improved cloning method

by somatic cell nuclear transfer from genetically designed cells. Both transposon-based gene transfer and knock out by homologous recombination with an AAV-vector system have been performed. We now have breeding colonies of pigs with some of the new genetic designs and crosses are performed to achieve more complex genotypes that should make the pigs prone to different disease processes like Alzheimer's neurodegeneration, atherosclerosis, psoriasis-like epidermal dysregulation etc. We have also created pigs with recombinant cassettes in the genome (to allow easy exchange of transgenes in open loci) and micropigs suitable for interventions in the gut microbiota. Sensor-reporter constructs for monitoring drug uptake and function have also been introduced. Danish HTF-funding is partially contributed by private enterprises (1/3) and spin off companies are formed. In China we have established a private, non-profit research organization (HuaDa JiYin/BGI) that now has become the largest genome centre in the world with many spin off companies. Experiences from these organizations will be discussed.

SP4.

Designing models for human cancer

Chris Counter, Duke University

Immunocompromised rodents engrafted with human cell lines and more recently genetically engineered mouse models of cancer have faithfully served for decades as the primary animal models of cancer for preclinical evaluation of therapeutics. Despite the freedom to manipulate genes, the low costs, large litter sizes, and short lifespan of mice, it has become apparent that rodent models do not always predict how therapeutics will act in human cancer clinical trials. Why this is the case likely varies from drug to drug, but arise due to genetic, anatomic, physiological and/or metabolic differences between the two species. This results in huge losses of time and expense in conducting clinical trials, not to mention the human cost. There thus is a need to develop new animal tumor models that better reflect human physiology and tumorigenesis. In this regard, the pig is genetically, anatomically, and physiologically more similar to humans, and metabolizes drugs and undergoes tumorigenesis akin to humans. Thus, the pig offers promise as an alternative model system that is more similar to humans to evaluate cancer therapeutics. As a first step to developing a porcine model of cancer, we demonstrated that porcine and human fibroblasts both require similar genetic changes to be converted to a tumorigenic state, namely the co-operative disruption of tumor suppressors and activation of oncogenes. Given this, we are in the midst of developing a transgenic pig in which an oncogenic mutant version of KRas and a dominant-negative version of the tumor suppressor p53 - genes commonly mutated in human cancers and is known to promote tumorigenesis of porcine fibroblasts - will be expressed in an inducible manner. Such a model will allow multiple tumor types to be generated depending on the tissue the transgenes are expressed. The development of this porcine cancer model will provide a critical reagent for studies dependant upon large animals or animals similar to humans, as well as lay the groundwork down for future collaborations with pharmaceutical companies to explore the use of this animal cancer model in preclinical evaluation of cancer therapeutics.

SP5

Modification of the male germline

I. Dobrinski

Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB T2N 4N1, Canada

Genetic modification of germline stem cells followed by germ cell transplantation (GCT) introduces genetic alterations prior to fertilization, does not involve embryo manipulation and decreases the time required to produce transgenic sperm. Genetically modified sperm from GCT has been used to generate transgenic rodents and goats. Male germline stem cells have been modified using viral and non-viral vectors in rodents with varying efficiency. Retroviral transduction requires replication

of cells in vitro and long-term culture conditions yet to be established for porcine germline stem cells. Adeno-associated viral vectors have proven successful in large animal models and exposed animals can be kept under standard husbandry conditions. Lentiviral transduction of porcine germ cells has been described, yet transgene transmission has so far not been reported. Germ cell nucleofection has also been explored, and initial results show that it can transduce primary germ cells in a goat model. Targeted modifications require selection and expansion of germ cells in vitro. Establishment of long-term cultures in mice has resulted in production of knock-out mice through GCT. However, progress in non-rodent germline stem cells has been slow. Increasing knowledge about species differences in the control of stem cell fate determination will provide the basis for establishing more appropriate culture conditions. Transposon mediated transduction, shown to be very efficient with rat GCT, as well as the more widespread availability of zinc finger nuclease technology will aid in overcoming the current shortcomings to generate targeted modifications in the porcine male germline. Supported by R01RR017359 and R41HD044780.

SP6.

Marketed drugs supported by the minipig as non-rodent species – review of FDA/EMA dossiers

Ganderup, NC

Ellegaard Göttingen Minipigs, Soroe Landevej 302, DK-4261 Dalmoose, email: ncg@minipigs.dk

Minipigs, like conventional pigs, are used as models of human disease and in safety studies of new medicines, medical devices, food additives and pesticides. This poster reviews the historical use of the minipig as a non-rodent species in regulatory toxicity testing to support new medicines and presents data on routes of administration, clinical indications as well as mechanism of action (where if possible) for more than forty marketed drug products. It also includes a qualitative assessment of the predictive value of the minipig by comparing adverse reactions in clinical trials and minipig studies. The most readily available source of comprehensive and unbiased information about licensed drugs is found in the publicly available databases of major regulatory agencies, where there are extensive non-clinical and clinical data from company submissions and it is critical assessment by independent experts. For the present analysis searches were made of two principal databases covering medicines registered in the USA, namely Drugs@FDA , and in the European Community EMA European Public Assessment Reports (EPARs); data mining and searches of said databases was done using PharmaPendium®. Such information pertaining to minipigs has, to the authors knowledge, never been published before, and is therefore seen as both new and ground breaking and surely warrants rethinking of the potential this species holds as non-rodent species in safety assessment. A more detailed account can be found in: The Minipig in Biomedical Research (2011, CRC Press/Taylor and Francis Group).

SP7.

Similarities and differences in academic and pharmaceutical research - exemplified by wound healing research

Peter Glerup

LAB Research (Scantox), Ejby, Denmark

Pharmaceutical research is usually a highly focussed process with many different departments working together with the same goal: to discover a new drug and to get a new product on the market as quickly as possible and (hopefully) before the competitors. Thus, the process is highly controlled, is planned in detail and has defined budgets available. The research has to fulfil certain requirements set by health authorities and all data is made available to the health authorities in relation to notification of clinical trials and at the final marketing authorisation application. In some cases the research may be published. In contrast, academic research relating to health and disease

often originates in political intentions for treatment of certain diseases or for safety protection of the general population towards hazards. Hence, public funding is made available for which research groups can apply. Another origin is private/charitable foundations with funding available for specific research topics and where research groups have to apply for grants. Academic research is often more diffuse, under less overall control and not performed according to the same level of quality assurance which applies to pharmaceutical research. Data is normally made publicly available by scientific publications. Despite the differences in the way the research is performed, pharmaceutical research and academic research should not be seen as conflicting. Actually, there is a high degree of dependency between the various type of research, with exchange of new ideas and methods. Seen from a Contract Research Organisation perspective, this presentation will discuss some of the elements mentioned above, exemplified in the area of non-clinical wound healing research.

SP8.

Pigs in dental research

S. Herring

University of Washington, Seattle

Pigs are remarkably similar to humans in the anatomy of the teeth and jaw muscles, salivary flow rate, the mechanics of the jaw joint, and the movements of mastication. Even the tongue and hyoid bone of pigs bear more similarity to those of humans than to other ungulates. The size and shape of the teeth and jaws have made pigs a favored model for testing clinical procedures such as dental implants and mandibular distraction osteogenesis. As in humans, there is a deciduous dentition which is very gradually replaced by permanent teeth, so pigs are also appropriate models for pediatric questions. Coupled with the enthusiasm of the animals for food, the study of chewing is easy. Some breeds of minipig suffer from severe malocclusions that resemble human disorders, although the genetics of such problems have not been elucidated. Pigs are also very subject to the accumulation of dental calculus and periodontal bone loss. Unfortunately, access to pigs is limited at many dental schools due to their urban locations and lack of facilities to handle large animals.

SP9.

The animal rule: developing a new medicine in minipigs without studies in patients - the ultimate test for any animal model

Stanley W. Hulet, PhD, DABT

US Army Edgewood Chemical Biological Center; Operational Toxicology; Aberdeen Proving Ground, MD.

The US Food and Drug Administration (FDA) have the obligation of protecting the public by assuring that new drugs approved for use are both safe and efficacious. The normal path for drug approval requires extensive testing in animals, as well as clinical trials in humans. However, what options do researchers have when they are investigating a drug intended as a treatment or prophylaxis to an extremely toxic chemical or biological agent? In 2002, the FDA issued regulations to allow the approval of drugs (21 CFR §314.610) and biological products (21 CFR §601.91) based on animal efficacy studies when human efficacy studies would be unethical or not feasible. The “animal rule” serves as a surrogate for human efficacy/clinical studies by allowing the potential for drug (e.g., countermeasure) approval based on efficacy studies in animals, coupled with the appropriate human safety and pharmacokinetic information. A caveat to the animal rule is that the FDA can rely on evidence from animal studies to determine the efficacy of the drug in question only when it is demonstrated that the animal model is expected to react with a response predictive for humans. Due to the many anatomical and physiological similarities between humans and swine, the minipig serves as an ideal animal model for predicting responses in humans.

SP10.***The pig (*Sus scrofa*) as a model for infant brain and cognitive development abstract***

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Use of the pig in neuroscience research is gaining popularity due to striking similarities between porcine and human neuroanatomy and similar patterns of perinatal brain growth, but few studies have employed this species for directly assessing brain and cognitive development. Nonetheless, the pig is well-suited for this application because as a precocial species, neonatal pigs can be artificially reared with relative ease and are amenable to behavioral training using a reward-based system at an early age. Thus, there is potential for the neonatal piglet to serve as a model for neuroscience studies focused on the impact of perinatal environmental stressors. To this end, our group has developed a suite of tools for investigating the impact of postnatal infection on brain and cognitive development in pigs. This includes (1) a novel technique for isolating microglia from discrete brain regions; (2) a magnetic resonance imaging protocol that we recently used in a longitudinal study that characterized brain growth (total brain volume, cortex, hippocampus, diencephalon, cerebellum, and brainstem regions) in male and female pigs from 2- to 24-weeks of age; (3) a protocol for analysis of neuron morphology from three-dimensional tracings made using Golgi-Cox staining procedures and NeuroLucida; and (4) behavioral tests that engage discrete brain regions (e.g., hippocampus) and assess cognitive plasticity. The ability to investigate the effects of infection on structural and functional brain plasticity in a preclinical translational model with brain growth and development similar to humans is of significant value.

SP11.***Coming of age: the pig as a biomedical model***

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Pigs display anatomical and physiological similarities to humans, and have drawn attention as a preclinical model for medical research. However, little has been reported regarding the scientific advantages of pigs as compared to those of dogs or non-human primates. At 2001, I surveyed all medical institutions in Japan that use pigs. The results clearly showed that there was scant available information regarding pigs and the support system for these animals as a biomedical model was poor. Then, I established the Tokyo Pig Project to study pigs as a biomedical model (Kobayashi E. 2002). In the present review, I discuss our 10 years of experience with this project and introduce our new pig research center, which began full operations in 2008. With dramatic improvements in pig genetic technology and somatic cloning technology, it is possible to say that pigs have become mature biomedical models.

SP12.***Combined SNP association study in human and pig related to subcutaneous fat thickness***

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Obesity represents a major public health problem by increasing risk to cardiovascular disease or metabolic disease. Although genome-wide association (GWA) studies in humans have expanded the number of genetic susceptibility loci for obesity, the variant detected in these large GWA studies explained only a small fraction of the inherited variability in non-syndromic obesity. Recently we

reported that neuronal genes for subcutaneous fat thickness in human and pig are identified by local genomic sequencing and combined SNP association study (Lee et al., 2011. PLoS ONE). To expand this local comparative association studies, we perform genome-wide SNP association studies to identify backfat thickness and intramuscular fat content in pigs. To relate the results observed in pigs to human common forms of obesity, we perform genomewide association study with subcutaneous fat thickness in a cohort population of Korean individuals.

SP13.

A joint funding opportunity on research in biomedicine and agriculture using agricultural animals

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In September, 2010, the National Institute of Food and Agriculture and the National Institute of Child Health and Human Development released the joint Funding Opportunity Announcement (FOA) titled “Dual Purpose with Dual Benefit: Research in Biomedicine and Agriculture Using Agriculturally Important Domestic Species” (see <http://grants.nih.gov/grants/guide/pa-files/PAR-10-276.html>). This FOA was released for three years with three funding cycles and submission dates (November 3, 2010; September 20, 2011; and September 20, 2011) and solicits R01 applications that are to be submitted to the NIH. The FOA was developed based on recommendations emanating from two stakeholder workshops held on the campuses of Michigan State University and the National Institutes of Health in 2004 and 2007, respectively. The primary goal of the FOA is to support high quality research that benefits both human health and animal agriculture. Additional goals are to increase the number of grant applications to the NIH that utilize agricultural animals, thereby increasing the need for reviewers having expertise with agricultural animals on NIH study sections. For applications to be competitive, applicants must: (1) address one of the areas of “Scientific Knowledge to be Achieved” identified in the FOA; (2) focus on a problem that is similar, if not identical, in human health and animal agriculture, and justify the project in terms of its relevance to human health and animal agriculture, and (3) use an agricultural animal as the model.

SP14.

Piglet model of pediatric nutrition and metabolism

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The perinatal period represents a very dynamic window of development for most mammalian species. Acute, late-stage development of the respiratory, circulatory, intestinal/digestive systems are critical to support extra-uterine life. Similarly, metabolism shifts abruptly from being largely carbohydrate-based in utero to being largely lipid-based during suckling. Given the need for these rapid adaptations, it is not surprising that neonatal morbidity and mortality rates are especially high. Our laboratory has developed and utilized the piglet model to study the postnatal development of fatty acid metabolism. Fatty acids supply 60% of energy for the postnatal piglet and bioactive eicosanoids derived from essential fatty acids regulate many important facets of cell growth, development and metabolism. Recent efforts have utilized PPAR α agonists to induce precocious expression of key enzymes regulating fatty acid oxidation such as carnitine palmitoyltransferase I (CPT1) and mitochondrial hydroxymethylglutarylCoA synthase (mHMGCS). Hepatic expression and activity of CPT1 can be elevated by 2-3 fold with similar increases in fatty acid beta-oxidation.

However, while mHMGCS message also can be induced, ketogenesis remains low. The potential of miRNA to modulate expression is currently being examined. Synthesis of long-chain polyunsaturated fatty acid (PUFA) also varies with early post-natal development, mediated in part by altered expression of delta-6 and delta-5 desaturases and elongase in liver and intestine, and expression depends on dietary PUFA content. Indeed, fundamental and pre-clinical research in the piglet model supported the FDA approval of PUFA supplementation into infant formula. Collectively this research demonstrates the versatility of the piglet as a pre-clinical model for infant nutrition and metabolism

SP15.

Transgenic swine 101. The fundamentals of a transgenic swine project.

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Advances in somatic cell nuclear transfer have greatly facilitated the development of genetically modified swine. This talk will cover the general aspects of the decision making process that takes place when designing a transgenic project in swine. In addition, we will cover some of the technical issues that need to be addressed as well as some of the most common problems that are encountered.

SP16.

Swine surgical models

Richard Pierson

University of Maryland

In addition to xenotransplantation models (which will be covered mainly by Dr. Robson), pigs are increasingly useful as a model for a variety of surgical investigations. Over 150 papers in the past 15 months report experimental surgery data based primarily on work in swine models. This presentation will review recent research reports where swine models were used successfully to study important surgical problems in organ and tissue allotransplantation, ischemia/ reperfusion injury, vascular surgery, shock and resuscitation, fascial or intestinal wound healing, medical education, skills training, surgical device testing, and development of new surgical procedures. The purpose of the talk will be to familiarize the audience with the variety of surgical tools that can be applied in porcine models, which can be leveraged using the expanding repertoire of genetic, molecular, and monitoring tools available to the modern translational investigator.

SP17.

Comparative phenomics: bioinformatic tools for utilizing the pig

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The sequencing of vertebrate genomes has democratized life science research. Genomic sequence is now the currency by which information about genetic mechanisms/function can be transferred and compared across species. However, to maximize the comparative power of the pig new tools/resources are needed to readily transfer biological information to and from other species. Toward this goal, a number of resources are currently under development. The swine research community has been working on the manual annotation of the swine genome, particularly gene structure and gene nomenclature (<http://vega.sanger.ac.uk/index.html>). A minimal set of information needed to describe QTL/association studies has been defined (MIQAS; <http://miqas.sourceforge.net/>). The Vertebrate Trait (VT) ontology in which trait nomenclature, trait definitions, and the relationship between traits are defined is currently under development.

Furthermore, additional ontologies (Measurement, Measurement Method, Product Trait ontologies) to help describe QTL/association data are also underdevelopment. The Animal QTL data base continues to grow and expand its functionality (<http://www.animalgenome.org/cgi-bin/QTLdb/index>). Finally, VCMaP, a tool that allows users to visualize orthologous genomic regions between Chicken, Cattle, Mouse, Pig, Rat, and Human genomes using genetic, genomic sequence and radiation hybrid map data has been developed (<http://bioneos.com/VCMaP/>). The VCMaP database contains an extensive set of quantitative trait loci (QTL) data and other annotations for each of these species. Furthermore, users can easily view private GWAS associations, SNP locations, Next-Gen sequencing, or any genome annotation. VCMaP tool has been implemented in Java and is launched via Java Web Start for complete platform independence and a responsive user interface. Taken together these resources/tools will help to facilitate the transfer of information between species. It is envisioned that these efforts will help elevate the visibility of swine as a comparative model for biomedical research model. Some of these efforts are supported by the USDA-NRI 2007-04187 and others by the NRSP-8 Bioinformatics Coordination project.

SP18.

Transgenic swine in xenotransplantation

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Xenotransplantation will only become clinically feasible once mechanisms of xenograft loss and rejection are better understood. The development of inbred miniature GalT-KO swine with removal of the dominant xeno-antigen has been a major advance but problems still persist in generating chimerism and obtaining tolerance to xenogeneic cells and vascularized xenografts. Further, thrombotic processes together with progressive xenograft microangiopathy and infarction remain prominent. These problems appear secondary, at least in part, to humoral immune reactions involving 'natural' or elicited nongalactosyl antibodies with complement activation. Xenograft rejection responses are further exacerbated by the documented intrinsic molecular incompatibilities in the vascular regulation of inflammation, complement activation, blood clotting and extracellular nucleotide homeostasis (i.e. purinergic signaling) between discordant species. Proposed strategies to tackle xenograft rejection include optimal immunosuppressive interventions, attempts to induce tolerance, judicious and more effective use of anti-thrombotics with further development of mutant and transgenic swine.

There has been significant progress in defining mechanisms of inflammation and coagulopathy linked to functional incompatibilities of CD39 and thrombomodulin across species, and determining novel vascular markers of injury associated with humoral rejection. A key goal is to limit xenograft injury and promote tolerance by targeting inflammation and innate immunity by swine transgenesis. We, and others, have proposed generating GalT-KO swine made transgenic for human complement regulatory proteins, anticoagulants and thromboregulatory factors (or null for defined porcine procoagulants). If successful, this approach will bring xenotransplantation closer to clinical application and provide insights into the regulation of thrombosis and vascular injury within transplanted organs. Furthermore, these studies also provide insights into the role of humoral inflammatory mediators, coagulation factors and platelets in the rejection of xenografts.

SP19.***Regulations and regulatory considerations*****Larisa Rudenko, PhD DABT and Malini Mansharamani, PhD**

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FDA regulates genetically engineered (GE) animals under the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act (FD&C) regardless of their intended use. Sponsors (producers of GE animals) also have responsibilities related to environmental assessments. Among others, GE animals are being developed for agricultural purposes (food or animal health), as sources of cells, tissues, and organs for xenotransplantation, to produce human (or animal) therapeutic agents (biopharm animals), and as models of human disease. Sponsors investigating GE animals, including species traditionally consumed as food (e.g., swine), are required to establish investigational new animal drug (INAD) files. FDA recommends that sponsors work with FDA to establish INAD files early in their research. INAD files are confidential mechanisms that provide the agency with information regarding the INAD and any associated investigations, and may also serve as a compilation of information that can be used to support future New Animal Drug Applications (NADAs) submitted by the sponsor. Sponsors have obligations and responsibilities relating to INADs, including record keeping, notification of shipping, appropriate labeling, identifying qualified investigators, appropriate disposition of investigational animals, and environmental assessments, including descriptions of how animals are contained and tracked. In general, the agency expects that all INAD files will mature into NADAs. CVM reviews data and information submitted for approval using a hierarchical risk-based approach. This includes descriptions of the method used to alter the structure or function of the resulting GE animal, characterization of the engineering event and its sequellae over multiple generations, phenotypic characterization of the resulting GE animals, and validation of the claims being made for the animal. The agency looks at the safety to the animal and of food from the animal (if intended for food use). In addition, the agency examines the potential for environmental impacts associated with the resulting GE animal. Sponsors should develop a durability plan to ensure that the genotype and phenotype of the GE animals remain stable over time. In addition, there are post-approval requirements including reporting the number of animals distributed, monitoring the stability of the genotype and phenotype, and any adverse events. CVM works with other FDA Centers responsible for approving products made by GE animals intended for use in humans (e.g., biologics produced by GE animals) with the goal of an overall streamlined, non-duplicative review process.

SP20.***The academic-corporate juncture--providing scientific evidence for an investigational new drug (IND)*****K. H. Schuleri**

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Adolescent farm pigs and adult minipigs are large enough to allow testing with human equipment and devices. With an increasing interest in translational research to move scientific result faster into the clinical arena without exposing patients to unnecessary risks, porcine models of MI and heart failure are most valuable in achieving fast IND approval for clinical testing. Cardiovascular imaging at present is considered crucial for better understanding and evaluation of the effects of new therapies and devices in the pre-clinical assessment. Magnetic resonance imaging (MRI) and computed tomography (CT) can be applied in preclinical porcine studies as cardiac imaging techniques for measurement of therapeutical effects of novel therapies. These imaging techniques are accepted to have accuracy and reliability for outcome and safety assessment, and are used as surrogate markers in preclinical and clinical studies. In recent years, myocardial transplantation of different cell types and preparations has been widely investigated as a potential therapy for

myocardial infarction (MI) and heart failure. We used porcine models to demonstrate that autologous and allogeneic mesenchymal stem cells (MSCs) can be safely delivered after acute MI and in heart failure, producing substantial structural and functional reverse remodeling. These findings demonstrated the safety and efficacy of cardiac MSC therapy and support currently ongoing clinical trials of cardiac MSC therapy.

SP21.

Porcine induced pluripotent stem cells for modeling regenerative medicine

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Porcine induced pluripotent cells (piPSC) were generated for modeling allogenic pluripotent stem cell and tissue transplants in large animal models. Generated piPSC chimeric offspring demonstrated that piPSC can contribute to the in vivo development of multiple tissues. However transplantation of undifferentiated piPSC into a disease model is unlikely, given the potential for teratomas formation in the recipient animals. Conditions for guided differentiation of pluripotent stem cells, including induced pluripotent stem cells, into progenitors that do not generate teratomas has been developed using human pluripotent stem cells in our laboratory. Shortly after we isolated three of the initial of NIH registered human embryonic stem cells lines we generated neural progenitor lines and used these to produce more uniformly differentiated cells ranging from motor neurons to dopaminergic neurons and now these have been successfully used in stroke and spinal cord injury animal models. Traditionally, in vitro differentiation processes for embryonic stem cells often utilize embryoid body formation because this is a relatively easy procedure, but rarely results in a uniform population of germ layer specific progenitor cells. We have developed more defined conditions required to individually produce uniform human neural, and now mesenchymal and germ cell specified progenitor cells in adherent human embryonic and now induced pluripotent ESC cultures without embryoid body formation. The guided differentiation requirements for these three specific lineage progenitor cells will be discussed.

SP22.

Construction of gene targeting vectors for use in porcine fetal fibroblasts

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Gene targeting has proven to be a robust technology in mice for gene evaluation and production of models. Essentially any genome modification that can be done in mice can now be accomplished in swine. With the completion of the porcine genome, vector assembly will generally be routine. Standard targeting vectors, promoter traps, and poly(A) traps have been used successfully to produce genetically modified pigs. At the National Swine Research and Resource Center an efficient system has been developed to simultaneously assemble targeting vectors and validate screening strategies. From project conception to gene targeted cells can be accomplished in as little as six weeks using standard molecular biology techniques.

SP23.

Use of adipose-derived stem cells in bone regeneration in a swine model

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Bone is a living polymer with substantial healing capacity. However, extensive bone loss due to disease or trauma may require the use of tissue-engineering methods. Presently, autogenous bone

grafting is the gold standard for bone repair, but presents limitations including donor site morbidity, bone shape, and amount. The use of stem cells appears to be a means to overcome such limitations. Bone marrow mesenchymal stem cells (BMSC) have been the choice, to date, for stem cell therapy for bone regeneration. Adipose-derived stem cells (ASC) are more abundant and accessible with lower donor site morbidity, making them a potentially better alternative to BMSC. Once ASC are obtained it is critical to establish a proper animal model that closely resembles the size of human bones for their use in pre-clinical trials. Among available animal models swine are the closest non-primate model for craniofacial configuration with two dentitions. Application of stem cells for regeneration of clinically relevant defects will require scaffolds that provide a nurturing environment, temporary function, replicate complex anatomic defects while being readily fixed to surrounding bone and be surgically implantable. The porcine animal model provides a valuable tool for scaffold use in tissue engineered bone regeneration with the use of ASC especially for complex anatomic defects in the craniofacial region.

SP24.

Zinc-finger nucleases: new innovations in custom-designed modification of the swine genome

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Genetically modified swine hold great promise in the fields of medicine and agriculture. By combining emerging gene modification technologies with the completion of the swine genome, custom-designed pigs will provide urgently needed organs for patients, realistic human disease models, and increased food production for the ever-expanding world population. Conventional gene targeting is inefficient in somatic cells with little control over the transgene integration site. The landscape of gene modification is changing as engineered zinc-finger nucleases (ZFNs) can enhance targeting efficiencies up to 10,000-fold. ZFNs consist of a nonspecific endonuclease domain and a sequence-specific DNA-binding domain. Specifically designed pairs of ZFNs heterodimerize upon binding DNA at predetermined gene loci, allowing formation of a catalytically active nuclease complex. Nuclease cleavage triggers DNA repair pathways that can disrupt gene coding or enhance insertion of exogenous DNA. Until recently, ZFNs have only been used to genetically alter small organisms. Publication of the first genetic modification in pigs by combining ZFN technology with somatic cell nuclear transfer has opened the door to genome targeting with a precision not previously possible in a large animal model. Subsequent reports describe PPAR γ mono-allelic knockout pigs and knockout of the α 1,3-galactosyltransferase gene in porcine fibroblast cells via ZFNs. The use of ZFNs has the potential to produce model gene knockout pigs without introducing any transgenic sequence into the genome. This presentation will provide an overview of ZFNs, current design methods, and innovative applications to accelerate the production of genetically modified pigs of agricultural and biomedical importance.

Session 1: Building Blocks of Models: Genomics, Bioinformatics, and Metabolism

S1-1

Determination of transgene integration loci by inverse PCR for multi-transgenic pig breeding

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Due to the limited efficiency of somatic cell nuclear transfer in the production of transgenic pigs, the regular supply of transgenic animals for experimental purposes requires the identification of a feasible breeding strategy for a multiple transgenic pig breeding herd that accounts for the conflicting issues of inbreeding and transgene segregation. Originating from two GalKO/CD46 transgenic founder boars, one human thrombomodulin (hTM) transgenic founder boar and three HLA-E transgenic sows, we have designed a breeding strategy that will lead to a core breeding herd of two GalKO/CD46 boars, two GalKO/HLA-E sows and five GalKO/hTM sows over two generations of initial breeding while at the same time allowing for later integration of further transgenes. In order to maximise breeding efficiency we have attempted to discriminate transgene zygosity in offspring. While the genomically assigned *GGTA1* locus allowed for the establishment of a duplex PCR that discriminates the wild-type from the knock-out allele, the other transgenes required prior clarification of the integration site. By optimising the inverse PCR method and performing two rounds of nested PCR on restriction digested and subsequently ligated genomic DNA of founder pigs we have so far been able to identify the integration site of the hTM transgene and establish a zygosity-specific PCR analogously to that for the *GGTA1* locus. Analyses of CD46 and HLA-E transgene integration sites are currently underway.

S1-2

Alpha-Galactosyltransferase (alpha 1,3-Gal) knockout genetically linked to xenograft transgenes

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Every year more than 100,000 people need organ transplants and in 2009 there were only 20,139 organ donors available. Genetically modified swine may be able to fill this unmet clinical need. It is clear that multiple transgenes will be required to make porcine organs or cells compatible with human recipients. Efficiencies in production of xenotransplantation pigs could be gained if multiple transgenes could be sequentially stacked at a single locus. The larger purpose of this project is to evaluate a site-specific recombination system as a tool to sequentially add multiple transgenes to the porcine alpha galactosyltransferase (alpha1,3-Gal) locus. One xenotransplantation related transgene, Human Decay Accelerating Factor (hDAF), is likely to be a part of any successful project since hDAF can prevent acute rejection by aiding in the deactivation of the complement system. To generate transgenic pigs that express an hDAF transgene that is genetically linked to an alpha1,3-Gal disruption, an alpha1,3-Gal gene targeting vector was constructed with homologous arms that were 4.8 kb (5'Arm) and 1.8 kb (3'Arm) and were derived from intron 8 and exon 9 of the porcine alpha Gal locus. Within exon 9, an IRES-Neo cassette and a CAG-hDAF cassette was inserted. A Phi-C31 AttB site was also included in the construct to later receive additional transgenes via a site-specific recombination system. Successful targeting of the alpha1,3-Gal gene has been achieved utilizing the described targeting vector and live animals have been produced from the targeted cells. The hDAF construct is highly expressed in all tissues examined.

S1-3

Unsorted adipose stem cells are more efficacious in bone healing compared to purified CD34+ cells

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In vitro growth characteristics and in vivo bone healing potential of porcine adipose stem cells (ADSC) unsorted or separated using the marker CD34 were performed. Aliquots of the ADSC were sorted into CD34 positive (CD34+) and CD34 negative (CD34-) cells. The unsorted ADSC (uADSC), plus the CD34+, CD34- , and a 1:1 mixture of CD34+ and CD34- (MIX) were differentiated in vitro into osteocytes. The number, dimension, density of bone nodules and alizarin red (AR) staining were quantified. Cells were harvested before plating then on several time points during expansion and differentiation for cells counting and RNA extraction. qPCR was performed for CD34, COL1A1, and SPARC genes. For the in vivo experiment, unsorted and sorted cells were transplanted, in duplicate, into 10 or 25 mm mandible osteotomies. Healing was evaluated by DEXA scanning after 8 weeks. In the in vitro trial the number of osteogenic nodules was higher in uADSC than the other cell types. The amount of AR was higher in uADSC compared to CD34- and MIX. Expression of all measured genes was similar between cell types. In the in vivo trial uADSC showed a greater healing compared to sorted cells. Overall data indicated that uADSC have a greater bone healing capacity than sorted cells.

S1-4

Characterization of normal skin thickness for various body regions, ages and genders of yucatan miniature swine

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Abstract This poster presents the results of a histologic skin characterization study in the Yucatan pig. Methods: Neck, dorsum or back (lumbar), flank, and abdominal skin were collected by 8-mm punch biopsy and immediately fixed in 10% neutral buffered formalin. The number of hairs per surface area was enumerated. The skin samples were then processed and stained with haematoxylin and eosin for histologic evaluation. Skin layers were measured microscopically using an image analyzing system. Each measurement was taken at random 5 times, then data were averaged. Four inter-follicular skin components (stratum corneum thickness, cellular epidermis thickness, number epidermal cell layers, and dermis thickness) were measured. Full thickness skin and full-thickness epidermis measurements were calculated. Relative ratios of epidermis, and dermis to full thickness measurements were calculated. Results: Across the 18 individual animals and all biopsy sites, the dermis averaged 3,305.33 μ M, the cellular epidermis 69.34 μ M, and the stratum corneum 29.10 μ M. Observed minimum and maximum values for measured components included dermis (587.29 μ M, 6,741.67 μ M), cellular epidermis (32.80 μ M, 140.93 μ M), and stratum corneum (6.23 μ M, 88.13 μ M). Female skin was thinner than male skin. Abdomen skin was thinner than the other sites evaluated. The cellular epidermis contained from 3-7 layers (mean 4.64 layers) and the trichogram from 0-39 hairs (mean 5.27 hairs) counted per 50.24 sq mm (8 mm diameter biopsy punch). Principal Conclusion: The differences in thickness for various Yucatan body sites is predominantly due to differences in dermis thickness as the dermis makes up 92-98% of full-thickness Yucatan Miniswine skin.

S1-5

AMPK mutation compounds ST segment elevation in the ECG of Ossabaw swine during cardiac ischemia

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Ossabaw swine are a highly translational model for ischemic heart disease. A spontaneous Val199→Ile mutation in the AMP-activated protein kinase (AMPK) γ 3 subunit of Ossabaw swine impairs AMPK function. AMPK normally functions as a metabolic sensor during conditions of cellular stress and affects many functions, including cardiac electrophysiology. We hypothesized that ischemia would induce greater ST segment elevation (STE) in the ECG of AMPK mutants compared to wild-type Ossabaw. Myocardial ischemia was induced by balloon occlusion of the circumflex artery and verified by angiography, intravascular pressure, and flow. Ischemia-induced STE was quantified as the ratio of STE over T wave amplitude on the ECG prior to ischemia. Lean AMPK mutants showed significant STE and lethal ventricular fibrillation within 3 minutes of ischemia, whereas wild-type swine showed only a 0.4 STE during 15 minutes of ischemia. Intracoronary infusion of the AMPK activator aminoimidazole carboxamide ribonucleotide (AICAR, 0.1 mM) for 10 minutes before coronary occlusion partially rescued the profound STE in lean AMPK mutants, with fatalities in 2 pigs by 7 minutes and 2 pigs survived 15 minutes of ischemia with 0.75 STE. STE was completely prevented in lean wild-type swine. During AICAR infusion 7 obese metabolic syndrome (“pre-diabetic”) mutants suffered lethal ventricular fibrillation within 7 minutes of ischemia. In contrast, 2 pre-diabetic wild-type had fatalities by 7 minutes and 1 wild-type survived 15 minutes of ischemia, which suggests a partial rescue of ECG function. We conclude that fully functional cardiac AMPK attenuates ischemia-induced STE myocardial infarction. (Support: NIH HL062552)

S1-6

Polymorphic patterns in toll-like receptor genes of suid species from different geographic locations

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Toll-like receptors (TLRs) are pattern recognition receptors that drive the very early stages of immune response in innate and acquired immunity. Polymorphism in TLR genes may influence their ligand binding or their signal transduction abilities and ultimately affect disease resistance in mammals. Differences in geographic locations are among factors suggested to drive polymorphism in TLR genes. In this study, we searched for Single nucleotide polymorphism (SNP), short insertions and deletions in the coding sequences of TLR1-10 of Suid species found in Asia and Africa. Read sequences from the Suid species were aligned to *Sus scrofa* reference genome using Mozaik as the read aligner. Gigabayes was used in calling polymorphisms. No polymorphism was observed in TLR3 and TLR4. SNPs were detected in all other TLR genes with TLR1 and TLR7 having the highest number of SNPs of 6 each and TLR9 having only 1 SNP. *Sus celebensis*, a Suid species from Asia had the highest number of SNPs as compared with *Sus verrucosus*, another Suid species from Asia and *Pharcochoerus africanus* of African origin. SNPs reported here will be investigated further in terms of amino acid substitutions in ligand binding and signalling domains in TLR genes.

S1-7

Site-directed modification of porcine genome by zinc-finger-nuclease technology

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Zinc-finger-nucleases (ZFN) are routinely used for genetic modification in various species. By usage of ZFN alone, site-directed but undefined mutations are introduced into the genome whereas a combination of ZFN with a targeting vector results in a site-directed modification possessing a desired structure.

In initial experiments we established proper culture conditions for ZFN-treatment of porcine primary cells, subsequent propagation of singularized cell clones and their screening for mutations at the target locus. In a proof of principle, we tested the efficiencies of different ZFNs binding to exon 1 in the CFTR gene. Sequencing of >100 singularized cell clones for each approach revealed an efficiency of 0-22% using either ZFN-coding plasmids or mRNA. In 14 of 74 mutated cell clones, two different modifications were found, indicating that both alleles are modified. The predominant type of mutations was gaps of a size between 2bp and 34bp. Rarely, inserts or local genomic rearrangements were obtained. Additionally, we established a targeting vector to introduce a lacZ-reporter gene under the control of the CFTR regulatory regions. The vector was co-transfected with a selected ZFN to generate a CFTR knock-in allele. Cell clones with a loss-of-function mutation or successful lacZ integration will be selected for somatic cell nuclear transfer and subsequent establishment of embryos for transfer into synchronized gilts. Thus, we present preliminary data on the suitability of ZFN to generate large animal models for biomedical research

S1-8

Clinical pathology parameters in Göttingen Minipigs – production herd data

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The use of minipigs in biomedical research has increased progressively with a significant surge over the past decade as evidenced by the very diverse areas in which they are being utilized. Reference data based on clinically healthy, normal animals is very important with the use of experimental animal models from both a veterinary and scientific perspective. Published normal ranges in clinical pathology can be used to help identify and diagnose disease, as well as differentiate treatment effects. This data is also very relevant when assessing the pharmacokinetic nature of a new chemical or therapeutic compound. This poster presents normal clinical chemistry, haematology, and coagulation parameters, as well as urinalysis, for the Göttingen Minipig based on representative samples from two breeding populations: one in Dalmose, Denmark and one in North Rose, New York, USA.

S1-9***Weight of select organs in Göttingen Minipigs – production herd data*****Ganderup, NC¹, Hayashi, N²**¹ Ellegaard Göttingen Minipigs, Soroë Landevej 302, DK-4261 Dalmose, email: ncg@minipigs.dk;² Oriental Yeast Company, 3-6-10, Azusawa, Itabashi-ku, 174-8505 Tokyo, email: nhayashi@oyc.co.jp

Minipigs are used to an increasing extent by the biomedical research community, a trend that has gained momentum the past ten to fifteen years. This increase in use amplified the request for background data which researchers can use to compare their data to. Their use includes both fundamental research and non-clinical safety assessment. Having this types of back ground information from the provider of animal in questions does not substitute for control groups or the added value of having in-house historical control data accessible, but is does offer an alternate source of this type of data. This poster presents weights (absolute and relative) of fifteen organ systems from both sexes (N=20-23) sampled at eight weeks and six months of age; the data are from the Göttingen Minipig breeding population (Dalmose, Denmark).

S1-10***Evolutionary analysis of Betaine Homocysteine Methyltransferase (BHMT) and BHMT-2 genes abstract*****R. S. Ganu¹, A. L. Roca², T. A. Garrow³ and L. B. Schook^{1,2}**¹ Division of Nutritional Sciences; ² Department of Animal Sciences, and; ³ Department of Food Science and Human Nutrition, University of Illinois at Urbana Champaign, Urbana IL 61801

The domestic pig is a model organism for research on the enzymes betaine homocysteine methyltransferase (BHMT) and BHMT-2, which convert homocysteine to methionine using the substrates betaine and S-methylmethionine, respectively. In order to identify conserved regions that could play a functional role in these proteins, sequences of the BHMT and BHMT-2 genes from 37 species of deuterostomes were compared. The BHMT gene was detected in sea urchin, fish, amphibian, reptile, bird and mammalian genomes. By contrast, the BHMT-2 gene was present only in the genomes of mammals, including monotreme, marsupial and placental species. Thus BHMT gene duplication occurred after the divergence of mammals from other living vertebrates, but prior to the divergence of extant mammalian subclasses. Across mammalian genomes, the BHMT and BHMT-2 genes were located in tandem on the same chromosome, though in all mammals the genes differed in regions that code for important functional and structural motifs. We identified seven codons that may have been targets of selection. A phylogeny of deuterostome BHMT and BHMT-2 sequences indicated that evolutionary rates were accelerated for BHMT-2 relative to BHMT. Comparing non-synonymous to synonymous mutation ratios across the phylogeny suggested that the highest levels of positive selection had occurred following gene duplication at the base of the mammalian clade. The patterns detected may be of potential relevance to the evolution of mammalian traits.

S1-11

MedSwine: A browser and genome portal for swine in biomedicine abstract

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The conservation of gene-function and physiology between people and pigs advocates for the utility of swine for modeling of human disease. Furthermore, large litters and advanced reproductive and genome-engineering technologies in swine provide efficient methods for the creation of large-animal models for biomedical and pharmacological research. The release of Sscr10 provides a valuable resource for guiding the engineering of the swine genome for the creation of these models. We have annotated the swine genome using a variety of resources, including: ENSEMBL annotation of pig genome build 9; our own annotation based on alignments of pig and human cDNA and protein; and ENSEMBL Gene Ontology annotations. We have also used sequence similarity to identify potential pig gene-targets by annotation transfer from the Online Mendelian Inheritance in Man and Human Phenotype Ontology databases. Annotation transfer from the Mouse and Rat Genome Databases provides an efficient method for understanding current animal models associated with swine/human gene orthologs. The MedSwine portal relies on two interfaces to facilitate querying these annotations: a GBrowse genome browser and a search interface that permits searching for genes, ontology, diseases, or by proximity to specific locations and features. Such a resource will facilitate the selection of gene targets and the development of molecular resources for genetic modification by homologous recombination. Furthermore, we demonstrate the utility of this resource by identifying intersections between swine genes, human disease genes, and putative target sites for an assortment of genome engineering tools, including the Sleeping Beauty transposon system, zinc finger nucleases, and meganucleases.

S1-12

A type I monoclonal antibody identifies a common epitope on multiple isoforms of the pig CD34

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CD34 is an important marker for the identification and enrichment of stem and progenitor cell subsets used in a variety of cell-based therapies but there are no commercial monoclonal antibodies available that cross-react with pig CD34 (pCD34). We have produced a monoclonal antibody which recognizes multiple isoforms of pig CD34. Balb/c mice were immunized with a fusion protein encompassing the extracellular region of porcine CD34. After screening a panel of 480 monoclonal antibodies we identified 1 antibody, 3G7, capable of recognizing porcine CD34 expressed in CHO cells, hematopoietic cells, adipose derived stromal vascular cells, and endothelial progenitor cells. The epitope recognized by 3G7 is sensitive to both glycoprotease and neuraminidase treatment, classifying 3G7 as a Type I CD34 antibody. We have previously identified multiple isoforms of porcine CD34 resulting from multiple single nucleotide polymorphisms in the CD34 gene. The 3G7 antibody was able to recognize two isoforms of pCD34 differing by 6 amino acids. Both isoforms had similar sensitivity to both glycoprotease and neuraminidase treatment. Western blot analysis of protein extracts from CD34-expressing CHO cells and enriched CD34+ primary cells demonstrates that pig CD34 expressed in these mammalian cells has an apparent electrophoretic mobility of approximately 100kD. The Type I 3G7 monoclonal antibody recognizes multiple isoforms of pCD34 providing a robust reagent to identify CD34+ cells in porcine tissues. This antibody will expand the potential of the swine model for use in preclinical trials of stem cell therapies.

S1-13

Multiple SNP variations occur in the porcine CD34 gene

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While stem cell-based therapies to address both hard and soft tissue regeneration have shown great promise in the pig model, the lack of stem cell reagents in the pig model makes it difficult to analyze stem cell populations used in this research. CD34 is widely used as a clinical marker for a subset of cells greatly enriched in hematopoietic stem cells. This marker is also associated with other stem and progenitor cells derived from adipose, endothelial progenitor cells, and skeletal muscle satellite cells. While variations in the CD34 sequence have been well studied in human and mouse, little work has been done to characterize variations in porcine CD34. We used mRNA from a single Yorkshire pig to amplify and sequence the open reading frame of porcine CD34. Using genomic DNA, the alternatively spliced Exon X was characterized. Based on sequencing of the first animal, PCR products were generated from the genomic DNA of 8 unrelated animals from each of the Sinclair, Hanford, and Yucatan minipig breeds. The frequency of single nucleotide polymorphisms (SNPs) in this sampling group was determined by sequencing the PCR products. A total of 15 SNPs were identified in exons 2, 3, 7, and 8 from the 25 animals tested. Of these SNPs, 8 resulted in changes in amino acid sequence. Three changes resulted in hydrophobic/polar shifts, one change resulted in a negative charge/polar shift, and one change alters a potential N-linked glycosylation site. Knowledge of potential variations in the translated CD34 protein will aid in the development of porcine stem cell reagents.

S1-14

Efficient targeting of genomic loci in the pig and production of knockout pigs by somatic cell nuclear transfer

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While site-directed modification is a routine procedure in mouse genetics, such approaches have been rarely accomplished in large animals due to the lack of embryonic stem cells. Conventional vector strategies suffer from a low rate of homologous recombination in primary cells and require adaptation for sufficient targeting. Extension of homologous arms might increase efficiency, but requires sophisticated screening tools. Here we present the suitability of BAC vectors for genetic modification of the pig genome in primary cells and their suitability as donor cells for nuclear transfer. We focused on the mutation of the porcine *CFTR*, *DMD* and *GGTA1* genes and initially modified BAC clones carrying the target region in the way we aimed to modify the genomic locus. These modified BAC clones were then transfected into pig primary cells and seeded onto 96 well plates to generate singularized clones with stable BAC integration. The clones were then screened for the loss of one wild-type allele of the respective target gene by qPCR and positive clones were used as donor cells for somatic cell nuclear transfer to produce mutant pigs. Targeting efficiencies ranged between 1.2% and 2.9% for all examined genes. Animals with null alleles for the X-chromosomal *DMD* as well as the autosomal *CFTR* genes have been produced by cloning. Thus, BAC vectors in combination with somatic cell nuclear transfer represent a powerful tool for the generation of site-directed mutations in large animal

S1-15

BWS symptoms in cloned piglets are associated with hypomethylation at the KCNQ-OT1 CpG Island

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Beckwith-Wiedeman Syndrome (BWS) is a loss of imprinting (LOI) condition that is associated with macroglossia, midline abdominal defects, and neonatal gigantism. A common feature of BWS is loss of methylation at the KCNQ-OT1 differentially methylated region (DMR). We hypothesized that this locus would show a similar LOI in cloned piglets displaying macroglossia. Sequence for porcine KCNQ-OT1 was assembled in silico. A CpG island was similarly located in porcine sequence as known for the human DMR. Primers were designed to amplify the DMR in bisulfite converted DNA and spanned 32 CpG sites. Imprinting KCNQ-OT1 in swine was confirmed. As seen in humans, this region was hypermethylated in half (12/24, $p=1$) of the cloned, sequenced amplicons. Next, two cloned piglets that appeared normal were assessed. DNA from each of these animals was consistent with normal methylation at this locus, (7/16 and 8/8 cloned, sequenced amplicons, $p>0.40$). Next, DNA was isolated from two cloned littermates where one piglet presented with macroglossia and the other did not. The non-presenting piglet's DNA was methylated in half of the amplicons (9/17, $p=0.67$) whereas the macroglossia piglet DNA was devoid of the methylation (0/14, $p<0.001$). An additional pair of macroglossia presenting and non-presenting cloned littermates was identified. In this pair, the non-presenting piglet showed a normal distribution of methylation (8/19, $p=0.77$) and the macroglossia piglet deviated from normal (6/20, $p<0.05$). These two case studies are consistent with the conclusion that the appearance of macroglossia in cloned pigs may be associated with hypomethylation at KCNQ-OT1 and may model BSW.

S1-16

Porcine ADSC and BMSC transcriptome during adipogenic and osteogenic differentiation

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In the present study we have directly compared the transcriptome of porcine adipose- (ADSC) and bone marrow- (BMSC) derived stem cells during in vitro osteogenic and adipogenic differentiation. At differentiation day 0, 2, 7, and 21 RNA was extracted for transcriptomics analysis by specific swine microarray. Data mining was performed by Ingenuity Pathway Analysis and DAVID. Using a False Discovery Rate <0.05 for overall tissue effect and a post-hoc correction of $p<0.001$ we observed 65 differentially expressed genes (DEG) between ADSC and BMSC prior differentiation. During differentiations we observed a lower numbers of DEG between cell types in osteogenic (<100 DEG) compared to adipogenic (<200 DEG) differentiation. Those DEG significantly enriched functions related to metabolism, antigen presentation, angiogenesis, and cell cycle. Overall, there was a greater induction of the enriched functions in ADSC and inhibition in BMSC during adipogenic differentiation and the opposite during osteogenic differentiation except for metabolism, which appeared to be larger in ADSC in all cases. The most significant enriched functions of DEG between the two differentiations in ADSC were metabolism, cell death, cell-to-cell signaling, and antigen presentation. In BMSC we observed enrichment of functions related to cell death, antigen presentation, and lipid metabolism. Overall, data uncovered a high similarity of the transcriptome between ADSC and BMSC both prior and during differentiations.

S1-17

Green fluorescent proteasomes in genetically engineered swine

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Proteasomes, protein complexes involved in protein degradation, undergo dynamic redistribution between cellular compartments. Characterization of the cellular localization of proteasomes is of interest for various physiological processes. We hypothesized that porcine proteasomes could be visualized *in vivo* via a ubiquitously expressed transgene encoding a proteasomal subunit (PSMA-1) fused to green fluorescent protein (GFP). Porcine PSMA-1 (20S proteasomal core subunit) sequence was assembled *in silico* from public data and used to identify an EST that appeared to be full length (CO946059). Primers were designed to remove the stop codon and create homology for cloning with InFusion (Clontech, Palo Alto, CA). The CO946059 amplicon was inserted into pCAG-CreGFP (Addgene, 13776) replacing the Cre coding region. The resulting plasmid (pKW14) was functionally validated in porcine fetal fibroblasts. After insert purification, PSMA-GFP, a selectable marker, and the chicken egg-white matrix attachment region were co-electroporated into male fetal fibroblasts (10ug total DNA, 5:2:2 ratio respectively). Transfected cells were cultured in DMEM (10% FBS) for 12 days in 400 mg/L G418. Embryos were reconstructed from pooled integration events via SCNT and transferred to two surrogates (120-125 couplets). Both females delivered two piglets each on day 114 by Caesarean section. One live piglet was produced from each litter. One survived beyond day 3 and continues to be healthy. Expression was confirmed by epifluorescence of GFP labeled proteasomes. This founder is being used to evaluate cellular localization of proteasomes *in vivo* and *in culture*.

S1-18

Metabolic consequences of low-linolenic acid soy oil in the Ossabaw Pig model of metabolic syndrome

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Consumption of trans fats is linked to obesity, systemic inflammation and other cardiometabolic diseases. It is established that incomplete hydrogenation of double bonds in vegetable oils such as soybean oil is a major factor responsible for the generation of trans fatty acids. Therefore, scientists have developed varieties of soybeans producing low-linolenic oil (LLO). However, the metabolic effects of the LLO have not been determined. Therefore, we evaluated the levels of selected serum metabolites and inflammatory responses of isolated adipocytes and monocytes from Ossabaw pigs fed either a diet supplemented with 13% regular soybean oil (SBO), LLO or a control diet (CON) for 8 weeks. Serum free fatty acid concentration was significantly higher in LLO fed animals compared to CON and SBO ($P < 0.05$). However, glucose and insulin levels were higher in SBO and CON groups than the LLO group ($P < 0.05$). When isolated monocytes and adipocytes were treated *in vitro* with lipopolysaccharide, the LLO group had reduced induction of TNF α and IL-6 gene expression ($P < 0.05$) compared to the SBO and CON fed animals. These findings suggest that a better metabolic profile and lower inflammatory outcome is associated with LLO consumption.

S1-19

Metabolomic footprinting of a transgenic pig model during the prediabetic state

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In general, the progression from prediabetes towards diabetes mellitus is diagnosed too late, when irreversible pathology like reduced beta-cell mass is already present. Therefore reliable markers indicating disease in the early stage are needed. A promising strategy of screening for such biomarkers is metabolomics of plasma samples in humans or relevant animal models. We performed a targeted metabolic study of transgenic pigs expressing a dominant-negative GIP-receptor (GIPRdn; Renner et al., *Diabetes* 59, 1228-1238, 2010) and of age-matched controls. Plasma samples from intravenous glucose tolerance tests (IVGTT, 11 measurements), which were performed in 11-week-old and 5-month-old animals (n=4 per group and age), were analyzed for 163 independent parameters (Biocrates). 11-week-old GIPRdn transgenic pigs show impaired incretin function but unaltered IVGT and beta-cell mass compared to controls. 5-month-old transgenic pigs exhibit a trend of impaired IVGT and a 35% reduced beta-cell mass. Metabolite values were statistically evaluated by analysis of variance (Proc Mixed; SAS) taking the effects of group, age, and group*age into account. Interestingly, 26/163 parameters were significantly ($p \leq 0.0001$) influenced by group*age and thus are potential markers for progression of the prediabetic state. Among them, the concentrations of 7/14 amino acids were increased in 11-week-old, but decreased in 5-month-old GIPRdn transgenic pigs vs. age-matched controls. Increased blood concentrations of amino acids were recently suggested as early predictors of diabetes in humans (Wang et al., *Nat Med* 17, 448–453, 2011), validating GIPRdn transgenic pigs as an excellent model for biomarker discovery in the context of a prediabetic state and its progression.

S1-20

Identification of Genomic Regions Associated with Cryptorchidism in Pigs

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Cryptorchidism, failure of one or both testes to descend into the scrotum, is a common abnormality in pigs and man. Research has shown that genetics contributes to the incidence of the defect. Unlike humans, porcine cryptorchidism is not typically associated with other physiological defects. Yet cryptorchidism does result in decreased value of animals and other production losses. A family of the U.S. Meat Animal Research Center's Landrace-Duroc-Yorkshire composite population with moderate inbreeding frequently produces cryptorchid pigs (34% incidence). In an attempt to identify genomic regions segregating with cryptorchidism, 12 affected, eight control siblings and all parents from this family were genotyped using the Illumina SNP60K BeadChip along with three affected animals and their parents from an unrelated litter. Association analyses were conducted with PLINK using the dfam option. Analyses were done with and without the three unrelated affected animals. A comparison of the results revealed that the unrelated animals reduced the significance values considerably, due to a different locus affecting cryptorchidism or different linkage disequilibrium between SNP markers and the causative genetic variation. Among the 28,304 mapped SNP markers that segregated within this family, numerous associations ($p < 0.006$ nominal significance) were found at SSC2:5.0-16.4 Mb (n=33) and SSC10:26.5-46.2Mb (n=68). An additional 136 affected, 222 controls and their parents (65 sires and 114 dams) were genotyped with 25 SSC2 and

10 SSC10 markers. The most significant marker was located at SSC10:27.4 Mb ($p < 0.005$). Additional markers are being tested and affected animals will be added as they are identified.

S1-21

Modulation of commensal gut and pulmonary microbiomes through oral microbial inoculation and its effects on systemic immune response

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The role of gastrointestinal microorganisms in developing and modulating the gastrointestinal immune response has been subject to investigation over the past few decades. However, early gastrointestinal tract stimulation and modulation of the systemic immune response is far from understood. This study explored the use of a non-pathogenic oral microbial inoculum to affect the composition of commensal microbiomes and subsequent systemic immune responses. Artificially raised piglets were divided into two groups: one group was orally inoculated (INOC) with non-pathogenic gut microbiota, and the second group was uninoculated (UNINOC). In order to determine the effects of changes in the microbiome on systemic immune response, allergic (type I) and delayed type (type IV) hypersensitivity responses were tested, as well as systemic immune responses via experimental infection with the respiratory pathogen *M. hyopneumoniae*. Pigs in group INOC had a lower severity of infection and stronger DTH response, suggesting a beneficial effect of the oral non-pathogenic microbial inoculation on commensal microbiomes and subsequent systemic immune responses. The 16S ribosomal RNA gene populations were amplified from DNA extracted from both fecal samples and nasal swabs collected temporally throughout the study. These amplicons were sequenced using 454 FLX-titanium technology to determine at great depth the effect of the oral microbial inoculum on both the gut and pulmonary commensal microbiomes. Preliminary analyses of the sequencing results reveal compositional differences in the pulmonary microbiomes between treatment groups, while analyses of the fecal microbiome are on-going. These results suggest the oral microbial inoculum is influential to our commensal microbiomes, and has a significant effect on systemic immune responses.

S1-22

Experimental infection of pig fetuses with E. coli

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The amniotic cavity creates relatively isolated microenvironment suitable for prenatal development of mammals. Its infection is extremely dangerous to both the mother and fetus and is the most common cause of premature labor. Increased levels of inflammatory cytokines also lead to damage of fetal membranes and trigger premature delivery. 10^6 CFU of enteropathogenic *E. coli* O55 bacteria was injected through the uterine wall of the gilt into amniotic fluid of pig fetuses (around 80 days of gestation). A surviving of pig fetuses and translocation of bacteria to adjacent amniotic cavity were estimated 10, 12 and 20 hours later. Increased time of infection was connected with high mortality of infected pig fetuses and bacterial translocation between the adjacent amniotic cavities. 10^2 , 10^4 or 10^6 CFU of *E. coli* O55 bacteria were injected into amniotic fluid. Amniotic fluid was aspirated ten hours later and bacteria were counted. The number of the counted bacterial CFU was not directly proportional to the number of the injected CFU. Levels of inflammatory cytokines IL-8, IL-10 and TNF- α in amniotic fluid and plasma significantly increased in the infected fetuses. This work was supported by grant No. 524/09/0365 of the Czech Science Foundation and the Institutional Research Concept AV0Z50200510 of the Institute of Microbiology.

S1-23

HMGB1 in the small intestine and plasma of gnotobiotic piglets

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High mobility group box 1 (HMGB1) is essential for correct DNA folding and transcription. It can be released from damaged cells or secreted by stimulated cells. HMGB1 has been detected in serum or plasma as a late mediator of sepsis. One-week-old germ-free piglets were orally infected with virulent *Salmonella Typhimurium* or *Escherichia coli* O55 or colonized with probiotic *E. coli* Nissle 1917 for 24 h. The transcriptions of HMGB1, IL-8, TNF-alpha, and IL-10 and their protein levels were determined. The piglets infected with enteric pathogens suffered from infections. HMGB1 was transcribed in the terminal ileum constitutively, regardless of any bacterial presence. In contrast, the transcription of cytokines was up-regulated by virulent bacteria. HMGB1, IL-8, and TNF-alpha levels in the ileum were increased by both enteric pathogens, while IL-10 levels increased in *E. coli* O55-infected piglets only. HMGB1 was significantly increased in the plasma of piglets infected with virulent *E. coli* only, but cytokine levels were in most cases increased by both virulent bacteria. HMGB1 and cytokine levels in ileum lavages and plasma of piglets colonized with probiotic *E. coli* remained comparable to those of the germ-free piglets. This work was supported by the grant ME 915 of the Ministry of Education, Youth and Sports of the Czech Republic and the Institutional Research Concept AV0Z50200510 of the Institute of Microbiology.

S1-24

Gene targeting of the swine GDF8 gene using AAV and TALENs

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The disruption of myostatin (GDF8), a negative regulator of muscle development and growth, causes enhanced muscle growth in numerous vertebrate species. In livestock this outcome results in elevated meat production and increased feed conversion efficiency. We hypothesize that inactivation of the pig GDF8 gene will induce hypermuscularity and an increase in feed convertibility. To facilitate public acceptance of genetically modified pork, we aim to disrupt the GDF8 locus using techniques that recapitulate naturally occurring alleles from other food animals, and which leave no ectopic DNA. We are pursuing two strategies to accomplish this; sequential gene targeting using recombinant Adeno-Associated Virus (rAAV), and one based on the induction of a double strand break using Transcriptional Activator Like Effector Nucleases (TALENs) coupled with template gene conversion. Putative clones from the first AAV targeting event have been generated and ready to be reprogrammed for the second targeting. This technique may be generally applicable to allele conversion of livestock genes for enhancing animal utility for both agricultural and medical applications.

S1-25

Disruption the Sialoadhesin and CD163 genes to create pigs resistant to PRRSV infectivity

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a viral pathogen in pigs. To better understand PRRSV infectivity and to potentially create a PRRSV resistant pig, two genetic modifications are being pursued. The first modification will disrupt a type 1 transmembrane glycoprotein that is a member of the sialic acid-binding lectin family which is expressed in

macrophages [Sialoadhesin (SIGLEC1)]. The second disruption will modify the N-terminal scavenger receptor cysteine-rich (SRCR) domain 5 of CD163, which is also a type 1 transmembrane glycoprotein. Modifications of these two genes are predicted to interrupt either PRRSV internalization (SIGLEC1 disruption) or viral disassembly in target cells (CD163 modification). A deletion from exon 1 through exon 3 has been introduced into SIGLEC1 in fetal fibroblasts. The modified fibroblasts were used for somatic cell nuclear transfer (SCNT) resulting in eight healthy piglets. Since previous reports have shown that a disruption/ modification of SRCR domain 5 of CD163 in cultured cells results in loss of PRRSV infectivity (J of Virology, 2010, p3101), a domain modification vector was chosen for CD163. For this targeting vector, exon 7 (SRCR domain 5) was modified to encode a peptide that is identical to human SRCR domain 8 of CD163-like protein (CD163L1). This CD163 modification vector will be used to transfect fetal fibroblasts. Targeted fibroblasts will be used to reconstruct embryos via SCNT. The combination of SIGLEC1 disruption and modification of SRCR domain 5 of CD163 is predicted to prevent PRRSV infectivity.

S1-26

DNA methylome status and cloning efficiency in swine nuclear transfer (NT) donor cells

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Successful cloning by somatic cell nuclear transfer is unpredictable due in part to abnormal DNA methylation that regulates gene expression in the NT donor cell genome. The objective of this study was to compare DNA methylation patterns in populations of swine fetal fibroblasts demonstrated to have either consistently high or low success at producing cloned pigs (HS; LS, respectively). We combined methylated DNA immunoprecipitation with high throughput sequencing (MeDIP-seq) to detect regions of methylated DNA in the two cell populations. A total of 272,598 and 261,339 genomic regions were enriched for methylated cytosine in HS versus LS cells, respectively. Many enriched regions (peaks) in HS and LS samples coincided in genomic position, but 12.9% were unique in HS cells versus 20.4% in LS cells. When intersected with predicted swine CpG islands, 3,129 of the total peaks (1.1%) overlapped for HS cells as compared to 1,836 peaks (0.7%) for the LS cells. These peaks were located in 1,505 unique locations for HS cells and 205 for LS cells, respectively. Chromosomal locations of the CpG intersections differed between each cell populations. Of the total peaks, 6,632 (2.4%) from the HS cell population overlapped with predicted swine genes compared to 4,041 (1.5%) from LS cells. Less similar was the percentage of these predicted genes that were unique to each cell group: 2,875 (43.4%) for HS cells versus 338 (8.3%) for LS cells. Comprehensive genomic analyses of epigenetic markers may provide predictive data to screen for optimal NT donor cells and improve cloning efficiency.

S1-27

Comparison of the epigenomes of pig, mouse and human pluripotent cells

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Epigenetic contribution to gene regulation, although important to animal development, aging and a variety of pathogeneses, is poorly studied in swine, an emerging animal model for biomedical research. To investigate the conservation and changes of the pig epigenome to the epigenomes in

humans and mice, we chose to compare pig induced pluripotent stem (iPS) cells to the pluripotent cells derived from humans and mice. On this end, we used chromatin immunoprecipitation followed by sequencing (ChIP-seq) and Methylated DNA immunoprecipitation sequencing (MeDIP-seq) to generate genome-wide maps of eight epigenetic markers (H2A.Z, H3K4me1, H3K4me2, H3K4me3, H3K9me3, H3K27me3, H3K36me3 and mC) and binding regions of two transcription regulatory proteins (TAF1, P300) in pig iPS cells. To compare these data across species, we first produced the same epigenomic maps in human and mouse embryonic stem (ES) cells, by compiling published data and generating new data whenever necessary. We then built a “Comparative Epigenome Browser” to visualize and compare multi-species epigenomic data in orthologous genomic regions. With these data and tools, we are starting to model epigenetic evolution, and to identify the essential epigenomic clues in regulating the pluripotent cellular state.

S1-28

Shifts in gut microbial community composition associated with degree of solubility of dietary fiber

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Trillions of microorganisms colonize the human gastrointestinal tract. They exert a strong influence on human health and diseases. Genes and diet are important factors in modifying gut microbial community composition. The role of genetics versus the role of shared diet, however, remains ill-defined. In this study, two genetically identical pigs in a withdrawal study of gut microbiome across four 14-d periods under two diets with different solubility of dietary fiber were used to eliminate the influence of genotype, isolating the role of diet as the main cause for difference in gut microbiome composition. In each period, the two pigs were maintained in the same environment and ate the same diet. Soybean hull diet, wheat bran diet, soybean hull diet, and wheat bran diet were provided in the first, second, third and fourth phases, respectively. Faecal microbial community of each pig under different diets was characterized using 454-pyrosequencing of amplicons from the hypervariable V3 region of the microbial 16S rRNA gene. Taxonomic classification and distance matrix was performed using Ribosomal Database Project classifier and compression-based distance, respectively. Taxonomy analysis exhibited that Firmicutes was the predominant phyla. Low solubility of dietary fiber increased Clostridia and decreased Spirochaetes at class level; raised Bacteroides, Oscillibacter and Succinivibrio and reduced Prevotella, Treponema and Escherichia at genus level. High solubility of dietary fiber played an opposite role in changing intestinal microbiome. Distance matrix analysis revealed that the individual bacterial community composition was distinctly clustered by the degree of solubility of dietary fiber.

S1-29

Increased propeptide processing of Factor IX in the milk of transgenic pigs by coexpression of Furin

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Coagulation Factor IX is a vital protein that plays an important intermediate role in the blood coagulation cascade. A mutation in the gene responsible for production of Factor IX results in

hemophilia type B. While the mammary gland can make many of the complex post-translational modifications necessary for biological function of blood proteins, improvements in propeptide cleavage and decreased proteolytic degradation are desirable. In order to explore the possibility of producing bioactive human Factor IX protein in pig milk, male Landrace fetal fibroblast cells were co-transfected by electroporation with Factor IX, Furin and SERPINA1. Tri-genic cells were used for somatic cell nuclear transfer producing fifteen F0 tri-genic male piglets from 3 different integration sites. Males were mated to wild-type females to produce F1 females. Tri-genic females were raised to sexually maturity then mated to a wild-type male. Tri-genic females were allowed to farrow and milk samples were collected for a 35 day lactation. Samples collected on Days 5-10 of lactation expressed FIX as well as no Pro-FIX, indicating that Furin activity was present. Additional Furin activity was detected on D35 of lactation. An aliquot of the tri-genic D35 milk sample was added to a mono-genic FIX milk sample, and after 1 h there was no detectable Pro-FIX. Two of the three male integration sites have been evaluated with similar results. We have shown the successful production of tri-transgenic swine for Factor IX production. Swine as Factor IX bioreactors have great potential to address current issues faced by hemophilia patients.

Session 2: Clinical Implications: Lesson from Successful Models

S2-1

Elevated renin and enhanced adrenal steroidogenesis in the Ossabaw miniature swine model of metabolic syndrome

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The Ossabaw pig fed atherogenic diet develops features of the metabolic syndrome (MetS). We hypothesized that development of MetS in Ossabaw swine would result in increased activation of the renin-angiotensin aldosterone system (RAAS) and development of hypertension. Male Ossabaw swine (~26 weeks age) were fed excess atherogenic diet for 55 weeks, resulting in robust MetS characterized by obesity, insulin resistance, dyslipidemia and hypertension. Serum creatinine and urea nitrogen levels were reduced in MetS vs lean lean controls (~13 and 48 %, $p < 0.05$), suggesting renal hyperfiltration. Plasma renin activity in fasting, unstressed animals was higher (0.5 ± 0.2 vs 2.9 ± 0.5 ng Ang I · ml⁻¹ · min⁻¹) in MetS pigs relative to lean controls. Aldosterone levels were also elevated ~6-fold in MetS pigs. An additional group of pigs fed atherogenic diet for 9 weeks was used to measure adrenal responsiveness in early MetS. Ang II (5 ng·kg⁻¹·min⁻¹) or ACTH (2 ng ·kg⁻¹·min⁻¹) infusion into conscious MetS pigs resulted in significantly greater peak aldosterone secretion relative to lean controls. Taken together, the data suggest that the development of MetS in Ossabaw pigs leads to an alteration in renal function and renin and aldosterone secretion are enhanced in this setting; the latter may be secondary to increased Ang II and possibly increased adrenocortical sensitivity. (Support: HL062552)

S2-2

Testosterone attenuates coronary artery disease in obese Ossabaw miniature swine

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Epidemiological studies in humans show a strong inverse association of serum testosterone with metabolic syndrome (MetS) and coronary artery disease (CAD), however, there are no prospective experimental studies. Ossabaw miniature swine develop MetS and CAD when fed excess calorie atherogenic diet. We tested the hypothesis that castrated male Ossabaws would develop greater MetS and CAD, thereby providing evidence for the protective role of testosterone. Eight castrated and eight sexually mature (age 6 months) intact males were fed the atherogenic diet for 29 weeks. At week 25 we performed MetS phenotype profiling and computed tomography (CT) to assess the body fat. The MetS profile included: blood pressure, intravenous glucose tolerance test (IVGTT), total cholesterol triglycerides, high and low density lipoprotein (HDL and LDL). Intravascular ultrasound (IVUS) imaging of the circumflex and left anterior descending arteries quantified CAD. The glucose tolerance of the castrated group was more impaired than the intact group. Also, the castrated group had higher cholesterol, LDL, and LDL/HDL ratio than the intact pigs. The intact pigs had higher body weights, greater lean mass in the CT images but no difference in the body measurements (e.g. girth) between the two groups, which supported the conclusion of greater adiposity in the castrated group. The IVUS imaging showed that castrated pigs developed more CAD than the intact group. We conclude that testosterone attenuated the development of MetS and CAD in the obese Ossabaw swine, thus providing an excellent model for study of testosterone therapies. (Support: Purina TestDiet, NIH HL062552)

S2-3

A model for identification of congenital defects in pigs

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Congenital defects can inhibit fetal development and survival. Due to morbidity and mortality in humans suffering from these defects, development of appropriate large animal models is important for advancing biomedical research. The objective of this project was to establish a model in which important human structural birth defects could be identified in large swine populations. Working in conjunction with a swine industry cooperator, the strategy was to dissect pigs that were either stillborn or dead within 48 hours of birth. This strategy minimized the number of dissections required while screening a large pig population (n = 51,397). In 2696 piglets dissected, defects identified include craniofacial clefting/cleft palate (n = 20), congenital diaphragmatic hernia (n = 12), congenital heart defect (n = 112), obstructive uropathy (n = 49) and spinal or limb defects, such as spina bifida (n = 18). Pedigree information was then utilized to identify and purchase animals that produced progeny with a greater than expected incidence of defects, as they would make ideal parents to generate the production of biomedical lines of animals. Narrow-sense heritability was estimated using variance decomposition implemented in the SOLAR software package and found to be significant only for heart defects ($h^2 = 0.31$, $P < 0.046$). In conclusion, our model allowed us to efficiently screen a large pig population and identify families with higher than expected rates of congenital defects to use for the production of animal models.

S2-4

Controlled gene expression in multi-transgenic pigs for xenotransplantation

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Successful development of somatic cell nuclear transfer (cloning) technology in pigs has allowed for precise genetic manipulation of the pig genome. For xenotransplantation applications, pigs have been produced in which both copies of the $\alpha 1,3$ -galactosyl transferase (GT) gene were inactivated (GTKO pigs). Analysis of tissues from GTKO pigs demonstrated a complete lack of immunogenic Gal $\alpha 1,3$ Gal (Gal) sugars, while in vivo pre-clinical studies in non-human primates, using cells (ie. pancreatic islets) or whole organs (heart, kidney, liver, lung), demonstrated the elimination of hyperacute rejection, and prolonged survival compared to wild-type controls. While survival of GTKO xenografts was extended, challenges including induced antibody responses to non-gal antigens, thrombosis, inflammation, and cell-mediated rejection remained, pointing to the need for further genetic modification of the source pig. Towards this goal, through a combination of cloning and breeding, in combination with GTKO, we have produced multi-transgenic pigs (some with 5 different transgenes) with controlled expression of genes for: i) complement regulation to address the humoral response to anti-non-gal targets (DAF, CD46); ii) inhibition of inflammation and thrombosis (TFPI, CD39, thrombomodulin); and iii) local protection against the human cellular response (CTLA4Ig, CIITA-DN). For some transgenes a constitutive promoter system can be used for expression in all tissues, such that one animal can be used for multiple transplant applications, however, our results have shown that for certain transgenes, tissue-specific gene expression is preferred. Since inhibition of thrombosis, complement modulation, and suppression of T cell responses are important to delayed xenograft rejection of both whole organs and islet cell xenografts, pigs have been produced with tissue-specific transgene expression in either the vascular endothelium or endocrine pancreas compartments, or constitutively in all tissues. In vivo results in non-human primates have demonstrated complete normalization of blood glucose for up

to 1 year in diabetic monkeys, and 8 month survival of multigenic pig hearts in baboons, evidence for the promise of this technology for human clinical applications.

S2-5

Amiodarone prevents fatal arrhythmias during complete occlusion of the circumflex coronary artery in Ossabaw swine

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The antiarrhythmic agent Amiodarone, is effective at preventing fatal arrhythmias during coronary ischemia, in Ossabaw swine. Several animals not treated with Amiodarone had their circumflex coronary arteries occluded and demonstrated a fairly rapid progression of arrhythmias: S-T segment elevation, premature ventricular contractions, ventricular tachycardia and ventricular fibrillation within 2-10 minutes of occlusion. Animals treated with Amiodarone demonstrated a robust resistance to arrhythmias; even when the occlusion was maintained for 75 minutes. While Amiodarone prevents those arrhythmias associated with sudden cardiac death, it does not prevent ischemic or infarctive damage to the myocardium.

S2-6

A porcine model of hereditary sensorineural hearing loss

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Chinese native pigs harbour rich spontaneous mutants with similar disease phenotype as human. Here we present a hereditary hearing loss family in Chinese Rongchang pig. ABR assessment on mutants at 1 to 60 days of age shows no signs of hearing indicating a profound congenital hearing impairment. SEM and Immunohistochemistry analysis indicated a progressive outer and inner hair cell degeneration in mutant cochlear, degeneration occurs in a basal to apical gradient and begins from about E80, no hair cells were detected on basal turn at postnatal day 1, eventually the near complete loss of hair cells in whole cochlear were displayed at postnatal days 60. A phenotype segregation family with three generations were constructed, and genome wide linkage and association analysis were used to mapping the mutant gene. A 24 cM linkage disease interval on SSC.13 were detected, and the strongest association signal were observed for two SNP markers in this interval (Log P=-8.77). A melanogenesis and hearing relate gene MITF were located between the two SNP markers, the mutation in human MITF gene result in the Waardenburg syndrome type II, it suggesting that hereditary hearing loss Rongchang pig is a model for studying the mechanism of Waardenburg syndrome type II and other hereditary sensorineural hearing loss.

S2-7

Development of a swine model of esophageal precancer

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Gastroesophageal Reflux Disease (GERD) is increasing in developed countries along with rising

obesity rates. GERD can progress to the metaplastic lesion known as Barrett's Esophagus (BE), and dysplastic BE can develop into esophageal adenocarcinoma (EAC), the fastest increasing malignancy in the Western world. Like esophageal squamous cell carcinoma, EAC is an aggressive cancer that now accounts for over half of the esophageal cancer deaths in the U.S. Although most existing animal models of GERD, BE and EAC have significant anatomic and physiologic differences from human, swine esophagus is anatomically, histologically and physiologically similar. Moreover, swine develop spontaneous nonglandular ulcers of the pars esophagus, underscoring the swine's potential for studying GERD, BE, and EAC. In the current study, 2/3 of the animals receiving daily oral deoxycholic acid (DOC) for 9 wk developed foci of atypical epithelium with some features of BE at the gastro-esophageal junction. The administration of a potent nitrosamine carcinogen (1/wk for 16 wk) increased lesion size and degree of cellular atypia in the group receiving DOC. We subsequently began administering DOC to the carcinogen-only group, and lesions became evident after only 5 weeks of treatment in these animals. Although the latency of potential nitrosamine-induced effects in swine esophagus is unknown, lesions failed to develop after 50 weeks in the carcinogen only group, whereas the DOC group developed atypical epithelial foci that were enhanced by carcinogen administration. Our overall intent is development of a preclinical model for studying mechanisms, prevention, early detection and treatment of GERD, BE, and EAC.

S2-8

Normal brain development of the domestic pig assessed by MRI

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The domestic pig, due to its anatomic and physiologic similarities to humans, is a well accepted preclinical model in cardiovascular, metabolic, and pediatric nutrition research and recently, appeal for the pig as a model for neurodevelopment research has increased. One limitation, however, is lack of normal brain growth trajectories from birth through adulthood in the pig. Anatomic MRI data were acquired using a three-dimensional T1-weighted MPRAGE sequence on a MAGNETOM Trio 3T imager. Two-dimensional images were manually segmented in each anatomical plane to permit brain region volume estimations. The normal growth patterns of total brain volume, cortex, hippocampus, diencephalon, cerebellum, and brainstem regions of 6 male and 9 female pigs were characterized from 2- to 24-weeks of age using seven MRI scans per pig in a longitudinal design. All brain areas increased between 110-150% in volume from 2- to 24-weeks of age. Logistical modeling of the data shows that there are sexually dimorphic brain growth patterns in different brain regions in both adult volume estimations and periods of maximum growth rate including the hippocampus, where males had a larger adult hippocampus which developed at a slower rate and later in life compared to females. Our results provide the normal growth patterns for the domestic pig, expanding the pig as a preclinical translational model for neurodevelopmental research.

S2-9

Targeted disruption of porcine LDLR: developing a model of hypercholesterolemia and atherosclerosis

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Atherosclerosis is the primary cause of cardiovascular disease, which is the most common cause of death in the United States. Atherosclerosis is characterized by the accumulation of lipids, cholesterol, calcium deposits, and cellular debris in vessel walls. This results in plaque formation, arterial obstruction, and diminished blood flow to organs. In time, these plaques can rupture and lead to thrombosis, resulting in myocardial infarction, stroke, or death. The main risk factors include elevated lipid levels, hypertension, and diabetes, which are heavily influenced by diet, lifestyle, and genetics. Several mouse models have been generated with mutations in genes important for lipoprotein metabolism, yet they fail to develop the complex atherosclerotic lesions that are typical of the human disease. In contrast to mice, the physiology and anatomy of the porcine cardiovascular system closely resembles that of humans. In fact, pigs have long been used as models of cardiovascular disease, and domestic pigs with naturally occurring low density lipoprotein receptor (LDLR) mutations and elevated serum LDL have been reported. While this hypercholesterolemic pig is a useful model, the wide disease variability, limited access by other researchers and expense of maintaining a large domestic pig prevent its wide use in the research community. Here, we report the development of male and female Yucatan miniature pigs with targeted disruptions of the endogenous LDLR gene using recombinant adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. Nine litters yielded 51 live LDLR-targeted pigs. Early phenotypic data is consistent with hypercholesterolemia. Additional details will be presented.

S2-10

Oncopigs as a superior model for breast cancer

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Currently, the efficiency of the identification of novel therapeutics for cancer is pitifully low. In large part, this is due to the failure of current animal models to be predictive for the human disease. Yet animal models are essential to develop drugs against metastatic cancer. Thus, we need a better animal model for cancer to fully exploit the rapid development of novel therapeutics and avoid a bottleneck in their development for use in humans. Current animal models are essentially limited to rodents, which differ genetically, biochemically, physiologically and anatomically from humans. Pigs, however, are very similar to humans in all these aspects by comparison. Indeed, the minimum genetic requirements for tumorigenic transformation in the pig appear to be almost identical to those required for humans. There is currently no transgenic pig model for human cancer. We are in the process of developing a transgenic "oncopig" model for breast cancer using breast specific induction of miRNA multimers in swine. This model could serve as a test bed for the development of novel breast cancer therapeutics and preventatives in a near-human system.

S2-11

Radiological, histological and molecular characterization of a swine HCC model

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This is the first report of a long term radiological, histopathological and molecular characterization of a swine HCC model. Swine liver cancer model was created in Yucatan minipigs by chemical carcinogenesis process. After administration of carcinogen (Diethyl Nitrosoamine), the liver lesions were observed in the CT images starting 7 months post carcinogen administration. Monthly CT, MRI and MR elastography were performed to characterize the development and progression of liver pathology including cirrhosis and tumors. MR elastography demonstrated development cirrhotic areas within 8-10 months post carcinogen administration. MRI and CT demonstrated heterogenous lesions in the livers of the pigs. Timed, image guided serial liver biopsies of the lesions demonstrated various types of liver pathology including necrosis, cirrhosis, focal nodular hyperplasia, adenoma and adenocarcinoma in the liver. The tumor lesions were positive for AFP (alpha-feto protein). Presence of liver cancer stem cell markers, including CD49f was detected in some of the carcinomas. Currently, efforts are underway to isolate and characterize as well as study the role of cancer stem cells in this model of swine hepatocellular carcinoma. Techniques to accelerate the carcinogenesis process also are being developed.

S2-12

Effect of dietary fat On adipose tissue gene expression and fatty acid composition of pigs

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The present study was conducted to assess whether the partial replacement feed energy by vegetable oils containing high Medium Chain Saturated Fatty Acids (MCFA) and n-6 Polyunsaturated Fatty Acids (PUFA) would modify adipose tissue lipogenic gene expression, fatty acid composition and serum lipid profile in pigs. The pigs were fed with an isocaloric diet in which 10% of the feed energy was replaced by either sunflower oil (SO) or coconut oil (CO) for 60 days. The dietary fat type had no effect ($P > 0.05$) on growth performance. The transcript concentration of lipogenic genes were reduced ($P < 0.05$) in adipose tissue of both SO and CO fed animals. A reduction in stearoyl CoA desaturase mRNA was observed in SO but not in CO fed pigs. Increasing trend in serum total cholesterol and adipocyte volume with insignificant changes ($P > 0.05$) in lipid profile was observed in both SO and CO animals. The adipose tissue fatty acid composition was significantly modified, with increase in MCFA and n-6 PUFA content in CO and SO fed animals respectively. Taken together, the results of present study indicate that the type of fat in diet can modulate adipose tissue gene expression and fatty acid composition differentially, with minimal effect on adipocyte size and serum lipid profile. The pigs may serve as an *in vivo* model in studying effect of dietary lipids in animal body.

S2-13

A porcine model studying prophylaxis and treatment of neonatal enteric disease with long-chain PUFA

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Dietary intervention is a potential therapy of enteric disease. Two objectives were investigated using *in vivo* and *in vitro* models of enteric disease. First, if supplementation of arachidonic acid (ARA) affects intestinal barrier repair in ischemic-injured ileum. Piglets consumed diets with 0%ARA, 0.5%ARA, 5%ARA, or 5% eicosapentaenoic acid (EPA). Following dietary enrichment, ileum segments were subjected to *in vivo* ischemia, and then control and ischemic loops were mounted on Ussing chambers. Transepithelial electrical resistance (TER) was measured over a recovery period. Ischemia-injured tissues from piglets fed the 5%ARA and 5%EPA exhibited enhanced recovery (% increase in TER = 13±13, 21.6±12, 59.1±12, 50.8±13, for 0%AA, 0.5%AA, 5%AA, and 5%EPA, respectively, p<0.05). Additionally, histology revealed reduced histological lesions of ischemic tissue from 5% ARA piglets versus other treatments (P<0.05). Second, if PUFA enrichment of flagellin-challenged neonatal enterocytes would alter proinflammatory response. Porcine jejunal epithelial cells were supplemented with 30µM PUFA for 96h. Cells were then stimulated with flagellin and cell RNA and media were harvested. Following supplementation ARA and EPA incorporation increased from 0.96 to 5.47 and 0.03 to 1.23 percent of FA, respectively (P<0.05). TNFα mRNA expression was numerically increased ~2-fold from 0-24h, and ARA and EPA treatment overall increased TNFα mRNA. However, there was a decrease in protein secretion from 0-24h (P<0.05), and ARA numerically decreased TNFα protein secretion compared to control (P=0.08). These data demonstrate increased supplementation of long-chain-PUFAs has a protective and therapeutic effect on ischemic-injured ileum, and potentially modulate proinflammatory immune response in epithelial cells.

S2-14

Pigs as a model to study functional genomics of selenium in energy metabolism, obesity, and diabetes

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Trace element Se and Se-dependent glutathione peroxidase-1 (GPX1) have been considered to protect against diabetes. Intriguingly, we found a spontaneous development of type 2 diabetes-like phenotypes in GPX1 overexpressing mice. Most striking, a number of major human studies have recently shown hyperglycemic, hyperlipidemic, and pro-diabetic effects of Se supplements. Because pigs are an excellent model for human nutrition, we have conducted a series of experiments to elucidate functional genomics of porcine selenoproteins in glucose and lipid metabolism. After identifying all 25 porcine selenoprotein genes using *in silico* cloning followed by PCR, we have determined effects of dietary Se concentrations on mRNA expression of these proteins in various tissues of pigs, and demonstrated three patterns of relationships between dietary Se level and selenoprotein gene expression. Subsequently, we have induced obesity and moderate insulin resistance in pigs by feeding a high-fat diet and monitored the expression profile of the 25 selenoproteins. The induced-obesity altered gene expression of 17 selenoproteins in various tissues of pigs. We have also conducted microarray analysis to explore systems biology related to body energy metabolism, selenogenome, and porcine genome. Our findings will help reveal novel metabolic roles of Se in energy metabolism, obesity, and diabetes.

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S2-15

A critical-size craniofacial bone defect model in the yorkshire pig

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Maxillofacial surgeons strive to develop the best therapy for large bone defects in the face resulting from tumor resection, congenital abnormalities and traumatic injuries. Creating a model to study a critical-sized bone defect in the mandible (one which does not spontaneously heal without clinical intervention) would be a method to test growth factors and synthetic bone graft therapies. However, the size of bone defect required to create this condition has not been ascertained. We examined the in vivo healing response for 4, 8 and 16 weeks of surgically created bone defects in the posterior region of the pig mandible. Yorkshire barrows (N=12) 6-7 months of age were used for the study. All animal experiments conformed to the University of Illinois Institutional Animal Care and Use Committee (IACUC) guidelines. Animals were maintained under general anesthesia and transcortical, circular defects with diameters of 6, 10, 16 or 25 mm were created on both sides of the mandible. The presence and amount of calcified tissue was assessed using radiographs and Dual Energy X-ray Absorptiometry (DEXA). Tissue morphology was examined using hard-tissue histological methods and a light microscope. In contrast to smaller defects, 25 mm diameter defects displayed limited collagenous tissue ingrowth, and calcified tissue was not detected. Defects in this region of the pig mandible with diameters equal or greater than 25 mm can be considered critical-sized defects. This porcine model will enable the development of new approaches for the repair of damaged bone, which is especially prevalent in the craniofacial area.

S2-16

Store-operated Ca²⁺ entry in coronary smooth muscle of the AMPK genotypes of Ossabaw miniature swine

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Calcium flux in coronary smooth muscle (CSM) cells is a major signal for excitation-contraction coupling and cell proliferation. Our previous study shows that metabolic syndrome (MetS; "pre-diabetic") Ossabaw swine with coronary artery disease (CAD) have store-operated Ca²⁺ entry (SOCE) in CSM. The SOCE channel is located in the sarcolemma and permits Ca²⁺ entry into the cell when activated by sarcoplasmic reticulum (SR) Ca²⁺ store depletion. Ossabaw swine have a spontaneous Val199→Ile mutation in the γ 3 subunit of AMP-activated protein kinase (AMPK) that impairs AMPK function. We compared SOCE differences in AMPK mutant and non-mutant Ossabaw on excess calorie atherogenic diet for 6 months. CSM were isolated enzymatically from coronary arteries and digitally imaged with fluorescent Ca²⁺ indicator fura-2. Ca²⁺ levels were expressed by the fluorescence ratio from 360/380nm excitation. Baseline Ca²⁺ levels were acquired by exposing cells to physiologic salt solution for 1 minute. We then depleted SR stores with caffeine, which binds to ryanodine receptors located in the SR membrane. The change in fura-2 ratio from baseline (ΔF) was measured after caffeine exposure and compared between groups. CSM from all pigs showed the classic rapid and high amplitude Ca²⁺ transient due to the rapid release from the SR. All animals displayed a sustained Ca²⁺ signal above baseline two minutes after continuous exposure to caffeine and depletion of SR Ca²⁺. The sustained Ca²⁺ signal serves as an index of SOCE. MetS mutant Ossabaw swine exhibited ~1.5-fold greater SOCE than lean mutants. There was no difference between MetS and lean non-mutants. (Support: NIH HL062552)

S2-17

Characterization of specific congenital anomalies of the urinary tract and diaphragm in swine

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Congenital anomalies of the urinary tract and the diaphragm cause high mortality and long term morbidity in humans. Obstructive urinary tract defects impair renal development in the fetus and newborn, and they are the primary cause of infant renal failure. Congenital diaphragmatic defects are associated with severe pulmonary hypoplasia causing respiratory distress and cardiopulmonary failure at birth. Although fetal intervention is a promising approach to improving outcome, development of fetal therapy or improved neonatal treatment is severely limited by the lack of appropriate large animal models. In a large scale perinatal lethal screen of piglets (in collaboration with North Carolina State University, Pig Improvement Company, and the U.S. Meat Animal Research Center), spontaneous but recurrent congenital anomalies similar to those found in humans were ascertained and characterized to determine suitability as human biomedical research models. The majority of urinary tract defects identified (n=39) mimicked those found in human neonates. Of these, 29 had diffuse ureteral dilatation, 14 had ureteropelvic junction obstruction defects, 10 had renal agenesis, 9 had megabladder without other identified anomalies, 3 had sacral dysgenesis, and 1 had a duplicated system. Congenital diaphragmatic defects were identified in 22 piglets. Of these, 10 were associated with additional anomalies. Posterior communicating defects were identical in morphology to severe human congenital diaphragmatic defects and were associated with severe pulmonary hypoplasia. Recurrence of human like congenital birth defects in specific swine herds is promising as a first step towards developing research models.

S2-18

Phenotypic characterization of diabetic INSC94Y transgenic pigs

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Different missense mutations in the insulin gene have recently been described as a cause of non-autoimmune permanent neonatal or infancy-onset diabetes in humans. In order to generate a large animal model resembling a human *INS* mutation and the *Ins2* mutation of the Akita mouse model, we transfected porcine fetal fibroblasts with a porcine *INSC94Y* expression vector (provided by Dr. H. Flaswinkel) and generated pigs by somatic cell nuclear transfer from pools of stable transfected cell clones. In total, 7 transgenic founder boars were born. One founder showed elevated fasting blood glucose levels at the age of 85 days, further increasing over time. Under insulin treatment the boar developed nearly normal and could be mated to a non-transgenic sow. Transgenic F1-offspring developed the same diabetic phenotype and exhibited reduced growth. Diabetic cataract was regularly observed. The weight of most organs was reduced proportionately with body weight. However, the kidney weight-to-body weight ratio (+29%; p<0.001) and the glomerular volume-to-body weight ratio (+23%; p=0.01) were significantly increased in transgenic vs. wild-type pigs at the age of 4.5 months (n=7 per group). Although no histopathological alterations of the kidneys were

observed, the elevated relative kidney weight and relative glomerular volume might represent very initial stages of nephropathy. This is supported by the observation, that the diabetic founder boar revealed unselective proteinuria at the age of 1.6 years. Quantitativestereological analyses of the pancreata from the 4.5-month-old animals revealed an 86% reduction ($p < 0.0001$) of beta-cell mass, consistent with the detrimental effect of mutant insulin.

S2-19

Transgenic pigs expressing the mutant insulin C93S for the study of pancreatic beta cell dysfunction

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Different missense mutations in the insulin gene revealed a divergence in findings at the molecular and cellular level with the clinical presentation and the severity of the disease in humans. In order to establish large animal models with varying degrees of beta cell dysfunction we have generated transgenic pigs expressing the mutant insulin C93S in addition to the previously established *INS*^{C94Y} transgenic pig model (unpublished data). The transgene consisted of German Landrace insulin sequences including 1.3 kb of the insulin promoter and 1 kb insulin gene sequences with the three exons and the T to A (C93S) point mutation in exon 3 analogous to the mutant insulin of the Munich *Ins2*^{C95S} mutant mouse model. Pools of stable transfected cell clones were used for somatic cell nuclear transfer. Five transgenic male founders were born. Disturbed intravenous glucose tolerance and reduced insulin secretion were detected in two founder boars at the age of eight months. The area under the glucose curve was 44%/31% larger, the area under the insulin curve 66%/64% smaller in founders 9748 and 9776 compared to controls respectively. This observation could be confirmed in transgenic F1-offspring of founders 9748 and 9776 at the age of 3 months ($p < 0.01$ for AUC glucose) and 7 months ($p < 0.01$ for AUC glucose). However, offspring from founder 9776 showed more serious glucose intolerance leading to the development of stable hyperglycemia in two sows from the age of 3 months onwards. Ongoing analyses comprise histopathologic analysis of the pancreas from offspring of both founders.

S2-20

Modulation of systemic immune response through commensal gut microbiota

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This study explored the use of a non-pathogenic oral microbiota inoculum to affect systemic immune responses. Artificially raised piglets (12) were divided into 2 groups. At four weeks of age one group was orally inoculated (INOC) with non-pathogenic gut microbiota, and the second group was uninoculated (UNINOC). Allergic (type I) and delayed type (type IV) hypersensitivity responses were tested by *A. suum* skin testing. Delayed type hypersensitivity (DTH) responses were stronger in the INOC group compared to the UNINOC group ($p = 0.07$). Four weeks after the oral inoculation, pigs were experimentally infected with *M. hyopneumoniae*. All pigs tested serologically negative to *M. hyopneumoniae*, porcine respiratory and reproductive virus and swine influenza virus at the beginning of the study, and remained sero-negative to both viruses throughout the study. Group INOC seroconverted to *M. hyopneumoniae* as early as 9 dpi, while all pigs seroconverted by 14 dpi. Onset of clinical signs also differed, being earlier for group UNINOC. The number of dry coughs suggestive of *M. hyopneumoniae* infection was greater ($p < 0.005$) in group UNINOC, and

group UNINOC tended to have greater ($p = 0.07$) lung lesion scores. No significant difference in cytokines, C reactive protein, bacterial load and TLR2 & TLR6 gene expression was detected between the two groups. However, a statistically significant difference in the variance of each group was seen for gene expression and some cytokine levels. These results suggest a beneficial effect of the oral non-pathogenic microbiota inoculation on the severity of disease outcome as demonstrated with mycoplasmal pneumonia.

S2-21

Combination of high fat and high sugar contributes to impaired glucose tolerance

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Pre-pubertal pigs may be a useful translational model for studying the physiological cues controlling childhood obesity. The objective of this study was to characterize changes in body composition and glucose metabolism in response to diets high in refined sugar, or saturated fat, or both. Female, weanling pigs ($n=24$, age=3 wk) were randomly assigned to control (CON), high fat (FAT; 15% fat), high sugar (SUG; 35% sugar), or high energy (HED; 15% fat, 35% refined sugar) diets for 16 wk. Ratio of ultrasound longissimus muscle depth to body weight was not different between treatments at any time point ($P>0.05$). However, by wk 4, backfat:body weight of FAT, SUG, and HED was increased ($P<0.05$) compared to controls. At wk 16, pigs were subjected to an oral glucose tolerance test (OGTT). Blood glucose of CON peaked at 45 min after oral dosing and returned to baseline ($P>0.05$) by 75 min post challenge. FAT and SUG response to OGTT were not different ($P>0.05$) from CON at any time point. However, blood glucose of HED pigs were greater ($P<0.05$) than that of CON pigs at 15 and 30 min and did not return to baseline until after 105 min after the challenge. These data show that consuming either high sugar, or high fat, or both results in increased fat deposition, but only the combination of fat and sugar results in impaired glucose clearance and suggest that control of glucose metabolism is dependent on the type of calories consumed rather than simply a function of amount.

S2-22

Surgical excision of coronary epicardial adipose provides evidence for its role in atherosclerosis

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Coronary artery disease (CAD) is the accumulation of atherosclerotic plaque within the lumen of large epicardial conduit arteries. There is a strong association between coronary epicardial adipose tissue (cEAT) and CAD. We tested the casual roles of cEAT in CAD by surgical excision of CEAT and longitudinal measures of CAD with intravascular ultrasound (IVUS). Ossabaw swine ($N=8$) were fed an atherogenic diet for 6 months to produce metabolic syndrome (MetS) and CAD. An adipectomy of the cEAT was performed in the proximal third of the left anterior descending (LAD) coronary artery, and the circumflex was a sham control. The pigs were allowed to recover on the same diet for 3 months. IVUS was performed during the adipectomy and sacrifice procedures in the LAD and circumflex to quantify atheroma. As expected there was an average increase in CAD during the 3 month recovery interval ($p<0.05$). The increase of atheroma in the proximal LAD was only 7% of that in the circumflex and the increase in the proximal and middle LAD combined was 38% of the same circumflex regions ($p<0.05$). In sharp contrast, the distal LAD segment increase in atheroma was 364% of the distal circumflex ($p<0.05$). We conclude that cEAT removal decreased the progression of coronary atherosclerosis. (Support: NIH HL062552)

S2-23

A validated swine model of cardiovascular disease for basic, translational and preclinical research

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The etiology of cardiovascular vascular disease (CVD) is multifactorial and dependent on the nuances of the interactions between mechanistic risk factors. Thus, the ideal model for CVD research must sufficiently reflect human genetics, physiology, and diet in order to reproduce the pathophysiological environment under which the disease initiates and propagates. While rodents have traditionally been the models for CVD research, challenges in translating rodent findings to humans due to differences in anatomy, lipid metabolism and gene expression have become increasingly clear. In contrast, the genetic proximity of the swine to human, and the similarities in energy homeostasis, adipose biology, lipoprotein metabolism, and cardiovascular system, highlight the suitability of swine for studies of human disease. In particular, our familial hypercholesterolemic (FH) swine model of spontaneous CVD has been extensively validated for studies of basic disease mechanisms and for the development of diagnostic and therapeutic technologies. The remarkable similarity of FH swine atherosclerosis to human atherosclerosis in lesion distribution, pathogenesis, and morphology, has been critical to our recent studies of adventitial vaso vasorum proliferation in early plaque development and vulnerability, and to the development of imaging modalities, including ultrasound and magnetic resonance imaging, for the early detection of CVD. In addition, the plasticity of the model in response to diet manipulation and the associated changes in metabolic regulation and adiposity emphasizes its value for translational research in obesity, metabolic syndrome, and CVD, especially in light of the increasing prevalence of childhood and adulthood obesity in the human population.

S2-24

Calorie-induced metabolic dysfunction in swine model of childhood obesity and cardiovascular disease

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The increasing prevalence of childhood obesity, and associated metabolic dysfunction, has significant implications on the early onset and severity of adulthood cardiovascular disease (CVD). A relevant translational animal model is needed to critically explore the mechanistic links between obesity, metabolic dysfunction and CVD. In our well-validated familial hypercholesterolemic (FH) swine model of spontaneous CVD, we examined the effects of calorie intake on adiposity and glucoregulation in order to substantiate the use of the swine model to study human obesity and CVD. FH swine were fed *ad libitum* (AL), 80% of AL or 60% of AL energy intake (N=6/diet) for 10 months beginning at weaning. All three diets were formulated to ensure that essential macronutrient and micronutrient requirements for normal growth and development were met. Over the course of the study, calorie intake significantly ($P<0.001$) influenced the rate and magnitude of body weight gain and change in percent body fat, as determined by dual-emission X-ray absorptiometry. At 10 months of age, intravenous glucose tolerance test (IVGTT) was performed to assess glucoregulatory function. IVGTT indicated significant differences in serum glucose clearance profiles and insulin sensitivity between the AL and 60% of AL fed animals; AL fed animals showed 5-fold lower insulin sensitivity ($P<0.05$) when compared to animals fed 60% of AL calorie intake. The results highlight the plasticity of the FH swine model to dietary manipulations and its potential for use in translational studies of CVD in context of obesity and metabolic dysfunction.

S2-25

The pig as an animal model for experimental maxillo-facial surgery

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Pig became recently popular as an experimental model for human craniofacial cancer research, tissue engineering or establishing new surgical techniques. Here, we aim to describe in detail the craniofacial anatomical structures of the pig, compare them to the human and so lay the groundwork for the practical clinical usage of the pig as an experimental model for craniofacial research. We selected diagnostic imaging methods, which are commonly used in human medicine and used X-ray examination, magnetic resonance and computed tomography for the morphological study in pig. Each of these designated methods exhibited advantages and disadvantages in case of their usage in pig model. We focused on the areas that are often affected by pathological processes (trauma, cancer, inflammation) in humans - orbit, oral cavity, nasal cavity, temporomandibular joint, maxillary and mandibular bones. Despite the porcine craniofacial structures share some histological and physiological similarities to the human, we found also significant morphological differences (e.g shape of temporomandibular joint, shape and number of teeth, number and position of mental nerves or maxillary sinus, shape of orbit, salivary gland position and number) that have to be taken into account before choosing the pig as an experimental model for the developing of new surgical techniques or for regenerative medicine. This work was supported by the Czech Science Foundation (grant 304/08/P289). The lab runs under IRP IPAG No. AVOZ 5045015.

S2-26

Development of genetically modified pigs suitable for diabetes and its complications research

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The present study aimed at identifying the characteristics of genetically modified diabetic pigs as a research model for diabetes and diabetic complications. A transgenic clone pig (founder, male) carrying a mutant gene, human HNF-1 alpha (P291fsinsC), responsible for maturity-onset diabetes of the young type 3, was created. The founder animal was treated daily with insulin to allow growth to sexual maturity, and then epididymal sperm were cryopreserved. Transgenic progeny obtained by artificial insemination of wild type sows with the cryopreserved sperm were pathologically examined. The transgenic progeny maintained a high blood glucose level (>200 mg/dl) through their growth period and their blood 1,5-anhydroglucitol concentrations were lower than those of healthy control pigs, indicating that they exhibited signs typical of diabetes. Also, the oral glucose tolerance test showed that the recovery of the blood sugar levels of the progeny was significantly delayed compared with healthy non-transgenic pigs. The hypoplasia of the islets of Langerhans was confirmed by the histopathological image of the pancreas, showing that the hyperglycemia of the progeny was ascribed to decreased insulin secretion. The histopathological image of the kidney showed a nodular lesion typical of diabetic nephropathy in humans. Also, vacuolated lens fibers,

suspected of cataracts, were confirmed in the equatorial region of the lens. These data demonstrate that the genetically modified pig that we created is a promising model for research into diabetes and its complications.

S2-27

Transgenic pig models for studying neurodegenerative diseases

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Transgenic animal models that express the disease genes have been widely used to investigate the pathogenesis of the diseases and to develop their treatment. Species differences, however, may be important for determining the nature of the pathology mediated by the disease genes. The fact that lack of overt neurodegeneration in transgenic mouse models for age-dependent neurological disorders such as Alzheimer's, Parkinson's, and polyglutamine diseases underscores the importance in establishing large mammalian animal models transgenic for these diseases. Pigs have been recently used to model genetic human diseases, because they are more similar to humans than mice in anatomy, neurobiology, life span and genetics. Using somatic cell nuclear transfer approaches, we have generated transgenic Tibetan miniature pigs that expressed the Huntington's disease protein, huntingtin, which carries an expanded polyglutamine repeat (105Q). Postnatal death, dyskinesia, and chorea-like movement were observed in some transgenic pigs expressing mutant huntingtin. Importantly, the transgenic HD pigs, unlike mice expressing the same transgene, displayed typical apoptotic neurons with DNA fragmentation in their brains. We are also generating transgenic pig models for other types of neurodegenerative diseases. These genetic pig models will be highly valuable for understanding disease mechanisms and developing therapeutics.

Session 3: War of the Worlds? The Academic---Regulatory --- Industry interface, a world of models, medicines, and scientific frontiers

S3-1

Stocking obese Ossabaw miniature swine at greater than biomedical research recommendations

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Obese Ossabaw miniature pigs are extremely sedentary and almost their only movement is to eat once per day. Current recommendations for the housing of swine for biomedical research funded by the National Institutes of Health (NIH) have no guidelines for obese swine that are so sedentary (Guide for the Care and Use of Laboratory Animals, 2010). We tested the hypothesis that obese (101-135 kg) Ossabaws, having minimal volitional movement, will show similar behavior, health, and physiological outcomes when housed at the recommended stocking density of 48 sq. ft./pig vs. the higher density of 24 sq. ft./pig. Ten obese adult Ossabaw pigs (age 12-16 months, body weight = 101-135 kg; ~2-fold >normal) were housed individually in pens at the higher density for 4 months and compared to 8 101-135 kg pigs housed at the recommended density. To comply with the animal welfare regulations regarding the floor space for swine in biomedical research we insured that at minimum the obese animal can turn around and express normal postural adjustments. We closely monitored all pigs during the study and at the end of the study we compared the following data: 1) glucose tolerance, 2) insulin resistance, 3) coronary artery disease, 4) blood pressure, 5) plasma lipids, 6) non-alcoholic steatohepatitis, and 7) blood chemistry. The health and physiological data showed no difference between 48 vs. 24 sq. ft./pig. We conclude that housing obese Ossabaw pigs (101-135 kg) at the higher density routinely should be acceptable practice to insure animal welfare (Support: HL062552, Amylin).

S3-2

Microminipigs: new SLA-defined pigs for biomedical research

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Despite the outstanding usefulness of miniature pigs as biomedical models to study human diseases, they have still disadvantages about breeding spaces, cost of feedstuffs, dose of test articles, and handlings of animals as compared with other large experimental animals such as Beagle dogs. To improve the disadvantages, miniature pigs designated as microminipigs (MMPs) in smaller body sizes were developed. A female miniature pig in an extremely small body size was derived as the initial MMP by the mating of a Pot-bellied pig and another breed of a miniature pig. A population of MMPs was established by successive breeding with the initial MMP. MMP was registered with the Japanese Ministry of Agriculture, Forestry and Fisheries as a novel variety of swine. The body size of MMP is much smaller than that of usual miniature pigs such as Göttingen and NIH pigs. Body weights of the young mature MMPs at 6 months of age are 7 to 8 kg, comparable to the weight of mature Beagle dogs. To establish swine leukocyte antigen (SLA)-defined MMP lines for the study of immune responses to allo- or xeno-grafts, we have assigned their SLA specificities by PCR-SSOP-Luminex and PCR-SBT methods. Seven and six SLA-DRB1 and -DQB1 alleles, respectively, including a novel allele in each locus were observed in the population. Selective breeding is ongoing in suitable allele combinations including novel alleles to select SLA homozygotes in MMPs individuals. The SLA-defined MMPs will be served as useful biomedical models of lifestyle-related diseases as well as transplantation studies.

S3-3

Book of normal data on selected lineages of miniature swine

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In an effort to further support biomedical research, we recently released a comprehensive dataset on normal data (reference intervals or ranges) for our four lineages of miniature swine. Included are Yucatan, Hanford, Sinclair S-1, and Micro-Yucatan lineages. This effort collates and summarizes normal biological and physiological data collected over many years. Data categories include: uses in biomedical research, clinical pathology (hematology, chemistry, coagulation, urinalysis), organ weights, growth, ECG, background histopathology lesions, blood glucose, ocular, diet/feeding, cardiovascular, dermal, reproduction data, and references. Over 80 tables of data are presented, along with most used citations and references. These data are offered to veterinarians, biomedical investigators, preclinical clients, and university staff to facilitate research when using our miniature swine animal models. This poster will outline the contents of this 'book' and present representative data tables. Copies of the full pdf on CDROM will be available by request.

S3-4

The RETHINK project on minipigs in the toxicity testing of new medicines and chemicals

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The objective of the RETHINK project was to evaluate the potential impact of toxicity testing in the minipig as an alternative approach in regulatory toxicity testing with focus on the 3Rs. Expert study groups (Working Groups) were assembled to review five different areas relating to the use of minipigs in regulatory safety testing: ethical issues, welfare and animal care, development of new medicines and chemicals, safety testing issues and emerging technologies in safety testing. It is concluded that there are no specific areas where restrictions to the use of minipigs in toxicology are required for welfare reasons. The minipig model is generally acceptable to regulatory authorities, provided it is adequately justified. The minipig is an interesting model for safety testing since there are numerous anatomical, physiological, genetic and biochemical similarities to humans. The use of the minipig in development of products does not bring any financial penalty in terms of the cost of testing. Benefits in terms of 3Rs can be identified. Finally the minipig (unlike the dog) is well positioned to take advantage of genomics and gene manipulation technologies. Further investigation needed to define the potential role of the minipig in testing of biologics (www.rethink-eu.dk).

S3-5

Use of pigs, dogs and NHPs in biomedical research in Canada, the European Union (EU), Japan, and USA

Niels-Christian Ganderup

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Data for pigs, dogs, and non-human primates (NHP) is reported for the regions and, where possible, area of scientific use is provided. The total use (all regions combined) of pigs and NHPs has increased (15% and 33% respectively) while the use of dogs has decreased (2%) from 2002 to 2008. There are striking differences between the patterns of use of the three species among

regions. Japan is unique in that dogs are used to a greater extent than pigs and NHPs. Dog use has decreased dramatically over two decades (38,915 in 1991 vs. 12,376 in 2007) and has not been replaced by pigs or NHPs. The USA has a constant use of the three species. Three observations can be made: (1) the use of dogs is fairly constant, (2) the use of pigs has gone down, (3) the use of NHP has increased. Canada and the EU have similar patterns of use of the three species. Pigs being used most frequently, followed by the dog and NHP. In the EU the use of pigs has increased and the use of dogs has decreased, while the use of NHPs is constant. The ratio P (p/d+nhp) of pig to dog and NHP varies with region and year: Canada [1.9-4.0], EU [1.4-3.0], Japan [0.01-0.16], USA [0.4-0.6]. E.g. for each one (1) combined dog and NHP 1.9 pig was used (Canada 2007). Thus, Canada and the EU has greater usage (and maybe acceptance) of the pig as a model in biomedical research.

S3-6

Marketed drugs supported by the minipig as non-rodent species – review of FDA/EA dossiers/NC

Niels-Christian Ganderup

Ellegaard Göttingen Minipigs, Soroe Landevej 302, DK-4261 Dalmoose

Minipigs, like conventional pigs, are used as models of human disease and in safety studies of new medicines, medical devices, food additives and pesticides. This poster reviews the historical use of the minipig as a non-rodent species in regulatory toxicity testing to support new medicines and presents data on routes of administration, clinical indications as well as mechanism of action (where if possible) for more than forty marketed drug products. It also includes a qualitative assessment of the predictive value of the minipig by comparing adverse reactions in clinical trials and minipig studies. The most readily available source of comprehensive and unbiased information about licensed drugs is found in the publicly available databases of major regulatory agencies, where there are extensive non-clinical and clinical data from company submissions and it is critical assessment by independent experts. For the present analysis searches were made of two principal databases covering medicines registered in the USA, namely Drugs@FDA, and in the European Community EMA European Public Assessment Reports (EPARs); data mining and searches of said databases was done using PharmaPendium®. Such information pertaining to minipigs has, to the authors knowledge, never been published before, and is therefore seen as both new and ground breaking and surely warrants rethinking of the potential this species holds as non-rodent species in safety assessment. A more detailed account can be found in: *The Minipig in Biomedical Research* (2011, CRC Press/Taylor and Francis Group).

S3-7

Minipig clinical pathology and urinalysis parameters in the European pharma-industry

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The use of minipigs in regulatory safety testing has increased significantly the past decade. With the increased use of minipigs establishing best practices for biomarkers (clinical-chemistry, haematology, coagulation parameters, and urinalysis) is essential to ensure predictive safety assessment studies, with patient safety and health as the ultimate goal. Much effort is invested in developing and validating biomarkers in minipigs and better understanding of current practices may prove more efficient and benefit both the researchers and research institutions. A survey of industry with the following objective was conducted:

- Collate information on current routine biomarkers and identify their value in the minipig.
- Identify biomarkers under development/consideration to address general and specific needs as markers of toxicity in the minipig.
- Learn what the minipig user community thinks the prospects and challenges are for developing and using new biomarkers in the minipig. Many standard biomarkers used in toxicology are established in minipigs.

Their development is primarily driven by the pharmaceutical industry's needs in regulatory safety assessment studies and while there is published literature on biomarkers available they do not necessarily meet the various needs of toxicologist. There seems to be an interest in creating a platform to share data and information about existing biomarkers as well as biomarkers under development. Such a database would benefit the use of minipigs in safety assessment as well as increase the accessibility and value of minipig biomarkers. This survey was conducted by Minipig Research Forum [MRF] Steering Group under the auspices of the MRF.

S3-8

Use of Göttingen Minipigs in reproductive and developmental studies—review of published control data

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According to ICH guidelines, and many regional and national regulations, new drug candidates intended for use in pregnant women or women of child bearing potential need to be evaluated for the potential to adversely affect pregnancy, and for any potential risk and hazard to the developing foetus. Fertility and early embryonic development (segment 1) studies look at potential effects on the reproductive organs, gametes and the embryo from mating through implantation. Embryo-foetal development (segment 2) studies evaluate risk to the developing foetus. Finally, post-natal (segment 3) studies will examine the effects on the offspring, as well as on lactation, up through weaning. Reproductive studies are routinely performed in a rodent and rabbit. However, in cases where the rabbit may not be suitable as the non-rodent model, the minipig may be a suitable alternative. This poster presents a review of the existing reproductive and developmental control data for Göttingen Minipigs. This data includes characteristics of the male and female Göttingen Minipig reproductive tracts, and embryonic development. Also included is background information about the nature and frequency of congenital malformations and abnormalities in the Göttingen Minipig, along with a comparison to other commonly used species for these types of studies. Although it cannot substitute for data obtained from individual control groups, this information can be valuable to help with the interpretation of reproductive or developmental studies which utilize the Göttingen Minipig.

S3-9

The use of minipigs in dermal and wound healing research

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The skin of pigs and minipigs shows many similarities to human skin. In drug development the most appropriate animal species should always be used for non-clinical safety testing, and for dermal pharmaceuticals it is therefore difficult to justify not to use the pig for these studies. But also in development of wound care pharmaceuticals and devices, the pig constitutes a much better model

than all other animal species. The wound healing process consists of the same phases as in humans and for both humans and pigs, wound contraction occurs quite differently as compared with loose skinned animals. In addition, the sensitivity of the skin of pigs is more similar to human skin, whereas exacerbated reactions are often seen in the rabbit. During drug development it is a requirement to test for local tolerance. In some instances this aspect can be integrated into single or repeat dose studies. However, if more detailed observations need to be made, including biopsy sampling at different time points in relation to dosing, separate studies with local tolerance as the major objective need to be performed. This presentation will focus on the use of minipigs in dermal- and wound healing research and especially in relation to regulatory requirements. In addition to this, an abraded skin model for use in regulatory toxicity testing of dermal products and other wound healing models will be presented. For each of the mentioned types of tests, scientific as well as practical aspects will be discussed.

S3-10

University of Illinois veterinary diagnostic laboratory supports swine research

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The Veterinary Diagnostic Laboratory (VDL), an AAVLD accredited laboratory, provides diagnostic medical testing and collaboration to support swine biomedical research as well as training for graduate students and researchers. The VDL can test for infectious and toxic agents, and other causes of disease in animals or diagnostic samples. The primary VDL location is at the College of Veterinary Medicine in Urbana, Illinois. The VDL is staffed by faculty experts in the fields of pathology, microbiology, virology, molecular technology, and parasitology with services offered in these areas. Pathology services include necropsy, histopathology (including immunohistochemistry), clinical chemistry and hematology with potential for GLP capability. Microbiology offers aerobic and anaerobic cultures, serology, PCR and/or FA for suspected organisms, antibiotic sensitivity, and mycology. Virology offers virus isolation, serology, PCR and/or FA for many organisms. Additional services offered include immunology/serology and molecular diagnostics. VDL faculties are also involved in research and can provide collaborative services. Their areas of interest include salmonellosis, mycotoxicoeses, and viral diseases. Previously faculty have collaborated with the College of Agricultural, Consumer and Environmental Sciences, Elanco, USDA and other research groups. Additional clinical expertise is available in the College of Veterinary Medicine. More information on the VDL is available at <http://vetmed.illinois.edu/vdl/index.html>. Inquiries can be made electronically at vldirectoroffice@vetmed.illinois.edu or by calling 217-333-1620.

S3-11

A piglet model for elucidating mechanisms governing dietary influences on iron nutrition

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Piglets and humans share many anatomical and physiological traits related to the absorption and utilization of dietary iron. We have exploited these similarities to study dietary factors that may enhance or inhibit the bioavailability of dietary iron and to explore mechanisms governing these effects. We have developed a hemoglobin repletion efficiency model for studying iron bioavailability from diets. In this model, we deplete piglets by reducing the amount of the iron injected at birth and then compare the effects of various dietary treatments on the rate of repletion of hemoglobin concentrations over 4 or 5 weeks. Using this model, we showed that supplemental dietary inulin enhances iron absorption in young pigs by affecting the expression of iron and inflammation related genes. In a related study, we tested the hypothesis that inulin enhances iron absorption by promoting iron absorption in the colon (it does not). In further studies of the effects of dietary inulin

in young pigs, we showed that inulin alters intestinal bacterial populations and hypothesized that this may partially account for its iron bioavailability-promoting effect and possibly other health benefits. We also showed that iron biofortified black beans provide more bioavailable iron to pigs than conventional black beans. Currently, we are studying the effects of tea ingestion on the secretion of proline rich proteins in the saliva as a possible mechanism for adapting to the iron absorption inhibiting effects of dietary polyphenols (supported in part by USDA-NRI and HarvestPlus).

S3-12

Comparison of agricultural and biomedical stocking density on growth and overall health of Ossabaw miniature swine

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Current recommendations for the housing of swine for biomedical research funded by the National Institutes of Health (NIH) are based upon “professional judgment and experience, and should be considered as recommendations” (Guide for the Care and Use of Laboratory Animals, 2010). To obtain more objective data on optimal stocking density for Ossabaw miniature swine we compared NIH, agricultural (Ag), and higher (>Ag) on growth and overall health. Standard animal husbandry applied, with the only difference being the stocking density. Pigs were placed in 1 of 3 groups of specific stocking density (sq ft/pig) at 1 month of age: NIH = 6, N = 15; Ag = 4, N = 22; >Ag = 3, N = 22). Pigs remained in these groups until reaching the average group weight of 25 kg, when the groups were spread out into the next stocking density requirements: NIH = 9, N = 5 and 10, Ag =, N = 22, >Ag = 5, N = 15). The study indicates when the pigs reached the average group weight of 50 kg. Growth rate, aggression, appetite, lameness, infections, abscesses were not different between the 3 groups. We conclude that Ossabaw swine are not negatively impacted by housing at a higher stocking density than current NIH requirements in the duration of this study. The broader implication is that the excellent pig health at higher stocking density would improve cost-effectiveness of swine for biomedical research. (Support: Purdue and IUSM Comparative Medicine Center)

S3-13

Impact of housing obese Ossabaw swine in a single versus double sized pen

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The obese Ossabaw is an excellent model for studying metabolic syndrome. It is not uncommon for these animals to reach over 100 kg, in which case it has been suggested that the health and welfare of these animals may be compromised when housed in individual pens. A study was conducted to compare changes in health status and well-being of obese Ossabaw swine weighing at least 100 kg and caged in either a single 24 x 24 ft pen or a double sized 48 x 48 ft pen. It was hypothesized that these obese swine would exhibit no signs of stress or discomfort when housed in a single 24 x 24 ft pen as there is typically a positive correlation between weight gain and degree of lethargy and inactivity. Multiple blood pressure and glucose tolerance measurements were collected to analyze any changes in health status. In addition, feed intake and overall stress levels were monitored daily to measure the well-being of each animal. No differences in blood pressure, glucose tolerance measurements, feed intake and overall stress levels were observed between the two groups of animals, suggesting that there are no adverse effects of housing obese Ossabaw swine in a 24 x 24 ft versus a 48 x 48 ft pen.

Session 4: Emerging Models: Regenerative Medicine, Behavior and Bioengineering

S4-1

Establishment of LEA29Y transgenic donor pigs for xenotransplantation

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Since T-cell activation is thought to play a major role in cellular rejection mechanisms, inhibition of the B7/CD28 co-stimulatory pathway by blocking agents such as LEA29Y might alleviate xenograft rejection. Thus, we established transgenic pigs that express LEA29Y specifically in the pancreatic islets to provide an experimental model for studying cellular rejection in xenotransplantation. The coding sequence of LEA29Y was placed under the control of the 1.3-kb core promoter of the porcine insulin gene and a polyadenylation signal was added from the bovine growth hormone gene. After transfection of the gene construct into porcine fetal fibroblasts, stably transgenic clones were pooled and used for nuclear transfer into enucleated oocytes. Following electrofusion and activation, the generated embryos were transferred to oestrus synchronised gilts. Two litters delivered a total of seven transgenic piglets of which each represented a unique founder, as could be demonstrated by Southern blotting. Four of these animals survived until an age of three months when they were sacrificed for immunohistochemical staining of organ spectra. The two piglets that displayed a strong, islet specific expression of the transgene product were chosen for re-cloning efforts to generate animals for breeding and for experimental purposes while establishment of a breeding herd is on-going. Ten re-cloned INS-LEA transgenic piglets were born out of five established pregnancies. Two piglets are being raised for breeding and will be mated within the next weeks while the others were utilised in pig-to-humanised-mouse islet transplantation to demonstrate functional capacity of the transgene.

S4-2

Systemic cytokine responses in swine following non-lethal staphylococcal enterotoxin B challenge

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Inadequate binding of superantigen-like toxins to class II MHC and T cell receptors limits the usefulness of rodents for modeling the human response to these noxious bacterial proteins. This fact, and the limitations inherent with human experimentation, remains an impediment for defining the deleterious consequences following exposure to these highly toxic superantigens. Developing therapeutic interventions or effective vaccines will require a valid animal model. To this end, we have developed a swine model to follow the acute, systemic cytokine response following in vivo challenge with a non-lethal dose of staphylococcal enterotoxin B (SEB). Unlike rodents, SEB can bind to, and effectively crosslink, pig class II MHC molecules and T cell receptors. Similar to the human disease, this binding triggers a toxin-mediated clinical response that can be difficult to measure. In an effort to identify parameters which would be easier to quantify, could be followed over time, and would correlate with the dose of SEB given, we measured several cytokines in sera after toxin challenge. Levels of IL-1 beta, IL-6, IL-8, IL-12p40, interferon-gamma, and TNF-alpha were measured at hourly intervals in response to varying doses of SEB given to individual pigs. We were able to demonstrate a pattern of cytokine production beginning as early as 1 to 2 hours post-challenge. Furthermore, such cytokine responses were ablated in pigs previously immunized against SEB. Taken together, these studies begin to define acute SEB-induced cytokine production,

and help establish a non-lethal model for evaluating therapeutics and vaccines useful for translation to human disease.

S4-3

Assessment of puberty in Mini-Yucatan boars by histologic evaluation and seminiferous tubule staging

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Stage of sexual maturity is an important factor to take into consideration when designing experimental protocols. While Mini-Yucatan boars are reported to reach “puberty” between 16 and 20 weeks of age, “puberty” can be defined in a number of different ways, such as a “threshold” portion of seminiferous tubules being actively engaged in spermatogenesis or the first appearance of epididymal sperm. Modified Davidson’s-fixed and PAS-stained testicular and epididymal sections were evaluated from 12-, 14-, 16-, 18-, 20-, 22-, and 24-week-old Mini-Yucatan boars (n= minimum of 4/age group). Approximately 200 seminiferous tubules were evaluated per testis for the presence of round spermatids only (immature tubules), as well as for species-specific cellular associations involving round and/or elongate spermatids (“mature” tubules) The “mature” tubules were “staged”, based on acrosomal morphology, and their proportion of the total number of seminiferous tubules evaluated calculated. The presence of sperm in the epididymides was also noted. Only the testes of one 12-week-old boar contained any spermatids (almost all round), while the testes of three of the four 14-week-old boars had seminiferous tubules containing elongate spermatids. Sparse numbers of sperm first appeared in the epididymides at 14 weeks of age (1 boar) and increased to at least three of six boars by 18 weeks of age. By 20 weeks of age almost all seminiferous tubules were “mature”, with sperm present in the epididymides. To the best of our knowledge, this is the first time that such histological data has been available for Mini-Yucatan boars.

S4-4

Potential benefits and limitations of porcine-to-murine testicular xenografts in toxicology studies

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Swine share many anatomic and physiologic similarities with humans and are likely to provide an accurate non-rodent model for human chemical exposures. The development of testicular tissue xenografting techniques using immunodeficient murine recipients addresses some of the potential logistical and financial concerns associated with porcine models, especially for preliminary studies. Testicular xenografting techniques can also provide additional opportunities for secondary manipulations of porcine testicular tissue during its postnatal development. However, there is a donor age, most likely around the time of puberty, when testicular xenografts are less likely to be successful. The objectives of these proof of principle experiments were to 1) demonstrate the potential use of porcine-to-murine xenografts (PMTXs) to assess the effects of xenobiotics on postnatal testicular development 2) evaluate the potential for PMTXs to undergo secondary manipulations and be successfully re-xenografted into murine recipients; and 3) determine the donor age when porcine testicular xenografts are unlikely to develop. Portions of testes from two, 13-week-old boars, orally exposed to either 0 or 100 mg VCZ/kg body weight were xenografted into castrated and vasectomized, immunodeficient mice (n=6 for each boar). The PMTXs from the untreated boar were larger and more steroidogenically competent than those originating from the VCZ-treated animal. In a second set of experiments, 14-week-old and, to a lesser degree, 24-week-old testicular xenografts originating from neonatal testes developed into functional testicular tissue after being re-xenografted. Testicular xenografts from boars older than 25 weeks of age were shown to be unlikely to develop into spermatogenically competent testicular xenografts.

S4-5

The generation of naive porcine induced pluripotent stem cells for genetic manipulation

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Although several groups have generated porcine pluripotent stem cells, the cells appear to share some features that hinder their use with genetic technologies including resistance to dissociated culture and limited capacity for chimera formation and germ-line contribution. Here, we are trying to capture more “naive” porcine induced pluripotent stem (iPS) cells. We prepared embryonic fibroblasts from Clawn miniature pigs (Japan Farm, Kagoshima, Japan) and introduced Yamanaka’s four genes (human Oct3/4, Sox2, Klf4, and c-Myc) with retroviral vectors into the cells. After cultivation with LIF and forskolin, emergent colonies were plucked and expanded for further analyses. The cells can differentiate into three germ layer cells in vivo (i.e. teratomas in immunodeficient mice) and in vitro. They express pluripotency markers and have sustained the normal karyotype. Similarly to mouse pluripotent stem cells, our porcine iPS cells form round colonies and grow rapidly. The naive state of our porcine iPS cells is indicated by LIF-dependency, two active X chromosomes (when female), and negative MHC class I. In addition, the cells are clonogenic: They can proliferate from single cells without a ROCK inhibitor. These results suggest that our porcine iPS cells are naive and highlight their potential as a tool for porcine genetic manipulation. Currently, we are testing the abilities to contribute to offspring chimeras by injection of porcine iPS cells into eggs.

S4-6

Characterization of porcine skin as a model for human skin studies using FT-IR spectroscopic imaging

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Porcine skin is often considered a substitute for human skin based on morphological and functional data, for example, for transdermal drug diffusion studies. A chemical, structural and temporal characterization of porcine skin in comparison to human skin will likely improve our understanding of this porcine skin model. Here we employ Fourier transform infrared (FT-IR) spectroscopic imaging to holistically measure chemical species as well as spatial structure as a function of time to characterize porcine skin as a model for human skin. Porcine skin was found to resemble human skin spectroscopically and differences are elucidated. Cryo-prepared fresh porcine skins samples prepared for spectroscopic imaging were found to be stable over the time and small variations are observed. Hence, we extended characterization to the use of this model for dynamic processes. In particular, the capacity and stability of this model in transdermal diffusion is examined. The spatial and temporal profiles of model compounds diffusing through porcine skin were determined by tracking characteristic spectral features of the compounds. The diffusion dynamics were shown to agree with previous reports. The results indicate that porcine skin is likely to be an attractive tool for studying diffusion dynamics of materials in human skin.

S4-7

Ultrasound strain imaging of arterial wall elastic parameters in a swine model of atherosclerosis

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The increasing prevalence of obesity, diabetes and metabolic syndrome in children and adolescents, presents increasing risk of early onset of cardiovascular disease (CVD) and occurrences of acute cardiovascular events. There is urgent need for diagnostic tools for early detection of CVD in pediatric population. Our familial hypercholesterolemic (FH) swine model of spontaneous CVD has been validated for systematic and reproducible in-vivo testing of emerging diagnostic and therapeutic ultrasound technologies. The development of vascular dysfunction at an early age (4-6 months) in FH swine precedes the development of visible fatty streaks (6-8months) and advanced lesions (>12months), and provides an ideal model for the development of early diagnostic technologies. The access to this unique translational animal model has allowed us to make great advances in developing methods of ultrasound strain and modulus imaging of arterial wall elastic parameters for the non-invasive identification of vascular dysfunction and changes in arterial stiffness as the earliest targetable event of cardiovascular disease. The novel scanning protocols and strain imaging algorithms that we develop will be clinically assessed at the UW Hospital and Clinics. The increased use of non-invasive ultrasound strain imaging will improve the overall efficiency and quality of early diagnosis of at-risk pediatric populations by preventative cardiologists.

S4-8

A challenge to developing humanized kidney using porcine renal anlagen as scaffold

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Producing organs in vitro from pluripotent stem cells is an ultimate goal in regenerative medicine. Use of animal anlagen as a scaffold or niche for stem cell growth is considered to be a promising option. We have been pursuing a strategy of regenerating a functional kidney derived from human pluripotent stem cells using developing pig metanephroi as a scaffold for organogenesis. This approach however involves generation of a chimeric kidney consisted of human and pig cells. In course of regenerating humanized kidney using pig metanephroi, it is highly demanded to eliminate the xenogenic components. In the present study, metanephroi (ca. 2mm in long-axis diameter) collected from E30 porcine fetuses produced by somatic cell cloning of fetal fibroblast cells were transplanted to the omentum of 4 adult cloned pigs with syngeneic background to the grafts to investigate their marginal development as a scaffold following ectopic transplantation. After 3 to 5 weeks, the transplanted metanephroi developed to vascularized kidney tissue of 5 to 20 mm in diameter composed of the renal pelvis, cortex, and glomeruli. Accumulation of urine accompanied with prominent ureter extension was also observed. Next, we created a transgenic pig fetus carrying mutant thymidylate kinase gene, and showed that the primary culture cells established from the fetus became apoptotic (Annexin V positive) in the presence of 0.5 to 5mM AZT. These

data suggest the possibility of developing porcine transgenic metanephroi which can be eliminated by inducible apoptosis after acting as a bio-scaffold for regeneration of humanized kidney.

S4-9

Porcine genomic domains orthologous to the human Prader-Willi syndrome chromosome region

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Prader-Willi syndrome (PWS) is a multisystem disorder caused by loss of function of a cluster of ~12 paternally-expressed, imprinted genes in human chromosome 15q11.2. Cardinal features of PWS include neonatal failure to thrive, abnormal body composition, childhood-onset hyperphagia and obesity, among other endocrine and behavioral abnormalities. As mouse models of PWS do not develop hyperphagia or obesity, alternative animal models are needed to study the biomedical bases and therapeutic approaches. Miniature pigs may provide an ideal model for PWS, since they have a more similar body size, physiology, and genome to human. While the pig genome sequence is close to being finished, nonetheless, the PWS-homologous region is poorly represented. Using sequence databases to screen for phylogenetically conserved sequences from the PWS domain, we generated *in silico* a BAC contig spanning large portions of the pig PWS-homologous imprinted and non-imprinted domains. Ten of the imprinted genes have been identified from partially sequenced BACs or ESTs. We identified unsequenced BAC clones that span the ~150-kb *cis*-acting imprinting center (IC) or extend from partially sequenced regions towards the IC. Several of these BACs are being sequenced by the Wellcome Trust Sanger Institute. Additionally, BAC clones for imprinted and non-imprinted regions are being FISH-mapped, with the IC-*Snurf-Snrpn* locus mapping to *Sus scrofa* chromosome 1q18. This work will identify the genetic structure, including imprinted genes, transcriptional and imprinting *cis*-regulatory elements, and the chromosome evolutionary breakpoints in the PWS-orthologous domain in pig, a region of significant biological, medical, and agricultural interest.

S4-10

Normal physiological ranges for hanford miniature swine

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The miniature swine have been increasingly recognized as a non-rodent model in regulatory toxicity. Members of the FDA have even published on the use of miniature swine as an alternative to canine and non-human primates in regulatory toxicity. The similarities between the cardiovascular, renal, and digestive systems make the miniature swine a suitable animal to model the human counterpart. The miniature swine are also the most recognized species for dermal toxicology. The Hanford miniature swine (HMS) has other attractive traits that make them a good substitute to model humans. They are omnivorous, easy to handle, prone to obesity, and will develop atherosclerosis and dyslipidemia if fed a high fat diet. With the advent of new techniques, all routes of compound administration can be used with miniature swine. The HMS should be considered as one of the non-rodent species in toxicity testing. In an effort to generate a database on baseline information about the normal physiological status of the Hanford miniature swine, we report expanded physiological data from normal intact and naïve juvenile and young adult miniature swine of both genders. The normal physiological data gathered includes growth parameters, hematology, serum chemistry, coagulation profile, urinalysis, ECG rhythm and segment intervals, and organ weights.

S4-11

Bioengineering hemostasis using recombinant human fibrinogen sealants in swine surgical models

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The treatment of shock trauma involving ensanguinating injury is particularly well modeled in swine surgical models. In addition to anatomical and physiological similarities, swine and human coagulation cascades are also very similar. We here describe the bioengineering of both recombinant human and plasma-derived fibrin sealant devices studied in pig liver lobe resection and laceration models. The recombinant human fibrinogen was made in the milk of transgenic cows. Nanomesh dressings in combination with recombinant fibrinogen, human thrombin (rFIIa) and Factor XIIIa (rFXIIIa) were designed to enhance wound specific adhesion while at the same time limiting exogenously applied fibrin to a layer that is less than 200 um. Immunohistochemical analysis of the treated wound topography showed an amalgam of adhesive, hemostatic structure at the interface between the exogenous human and endogenous pig fibrin. The recombinant fibrin sealant was hemostatically equivalent to human plasma derived sealant where rapid hemostasis was demonstrated in pig liver lobe wedge resections, lobectomy, and a grade V+ wound made by stellate liver laceration.

S4-12

Progressive rod degeneration in a miniature pig model of retinitis pigmentosa

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We have developed a model of retinitis pigmentosa (RP) using somatic cell nuclear transfer with the most common rhodopsin mutation in autosomal dominant RP (Pro23His) in an inbred miniature pig. The aim of this study is to characterize the progression of retinal degeneration in the P23H pig retina during the period of maximum photoreceptor degeneration. Pig eyes were examined and monitored using ophthalmoscopy, fundus photography, fluorescein angiography and OCT from postnatal day 1 (P1) to 18 months of age. Retinal degeneration was assessed by histology and immunocytochemistry of rod and cone photoreceptor markers. Photoreceptor cell apoptosis was examined using TUNNEL staining. Photoreceptor cell death was evident at P1, and OCT demonstrated marked retinal thinning at 21 months; H&E staining of retinal sections demonstrated that only one row of photoreceptors remained in the outer nuclear layer at this time. Immunostaining demonstrated a progressive loss of rod photoreceptors during the period between P1 and 18 months of age. And, by 21 months of age, cones comprised the single row of photoreceptors remaining. In conclusion, the P23H miniature pig displays progressive loss of rod photoreceptors mimicking what is observed in RP patients. Thus, these transgenic pigs represent a large animal model of rod photoreceptor loss which can be used in future studies of cell transplantation or other therapies for retinal repair.

S4-13***Pig iPSCs are capable of generating normal chimeric pigs and undergo neural differentiation in vitro*****Franklin D. West**^{1,2}, **Elizabeth W. Uhl**³, **Jeong Yeh Yang**^{1,2}, **Yangqing Lu**^{1,2} and **Steven L. Stice**^{1,2}.¹Regenerative Bioscience Center; ² Department of Animal and Dairy Science; ³ Department of Pathology, University of Georgia, Athens, GA 30602

Pigs are a desirable large animal model to study the efficacy and safety of induced pluripotent stem cell (iPSC) therapies for a number of diseases. However, in the mouse numerous tumors were formed in chimeric animals derived from iPSCs resulting in health problems and high mortality rates. This has raised significant questions pertaining to the tumorigenicity of iPSCs and whether data in the mouse will translate to other species and eventually human iPSC therapy. Our lab has for the first time generated chimeric pigs from pig iPSCs (piPSCs) that show iPSC contribution in all 3 germ layers. To begin to address the question of tumorigenicity and potential abnormal development resulting from iPSCs in a non-rodent model, we performed necropsy and histological analysis of collected tissues from chimeric pigs at 2, 7 and 9 months of age. Necropsy results and histological analysis showed that test animals demonstrated normal organ development and lacked tumor formation despite many tissues being comprised of piPSCs as indicated by the presents of the human POU5F1- a human gene utilized in the reprogramming of piPSCs. Ultimately for piPSCs to be beneficial in studying iPSC therapies, piPSCs must be capable of directed differentiation. Utilizing a neural differentiation system, piPSCs neural progenitor-like cells have been derived that express neuronal, astrocytic and oligodendritic markers and are capable of forming axon and dendritic like extensions. piPSCs that do not cause tumors and are capable of neural in vitro differentiation presents a powerful translational model to study the potentially of iPSC therapies.

Session 5: NSRRC Workshop on Developing Transgenic Pig Models

S5-1

Improving gene targeting efficiency by utilizing a splicing-dependent selectable marker

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Gene targeting in mammalian cells plays a crucial role in biotechnology. These experiments are characterized by low rates of homologous recombination and high rates of random integration. To dramatically reduce the survival of random integration events, we have developed a splicing-dependent selectable marker strategy by introducing a mutation in a codon-optimized G418 resistance. Since the C-terminal region of aminoglycoside phosphotransferase (AphII) participates in formation of the active site of this enzyme, we hypothesized that addition of even one amino acid at the C-terminus would render this protein non-functional. To test this hypothesis, a mutation was introduced in an E. coli AphII expression vector that converted the stop codon of AphII to tryptophan (X265W). This mutation was confirmed to inactivate AphII in E. coli. To evaluate this strategy in porcine fetal fibroblasts, two plasmids were constructed that harbored the X265W mutation embedded at the 5' splice site of a downstream intron. In one plasmid (pSC2-G) the first base of the downstream exon begins with a G residue resulting in inactivation of AphII, while the other plasmid (pSC2-A) harbors an A residue forming a stop codon (TGA) that allows for active AphII. A positive control plasmid and pSC2-A produced colonies that were too numerous to count. A negative control plasmid and pSC2-G produced no colonies. It can be concluded that the X265W mutation can be corrected by splicing to an exon that begins with an A residue.

S5-2

Quercetin improves in vitro development of porcine oocytes by acting as an antioxidant

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Quercetin is a plant-derived flavonoid that has antioxidant properties and acts as a free radical scavenger. We investigated the effects of quercetin on maturation of porcine oocytes and embryonic development when quercetin was added to medium during in vitro maturation (IVM) of oocytes and in vitro culture (IVC) of embryos. Then we evaluated the antioxidant effects of quercetin by measuring intracellular glutathione (GSH) levels and reactive oxygen species (ROS) levels during IVM and IVC. Immature oocytes were untreated or treated with 1 µg/ml quercetin during IVM and IVC, separately. Quercetin treatment did not improve nuclear maturation of oocytes, but a significantly greater proportion of parthenogenetically activated (PA) oocytes developed into blastocysts (15.8 vs. 9.8 %) when the IVM medium was supplemented with adequate quercetin (1 µg/ml); however, cleavage rate and blastocyst cell number were not affected. In PA embryos, quercetin treatment during IVC did not improve blastocyst formation rate, significantly. Quercetin-treated oocytes during IVM or IVC had significantly increased GSH levels and reduced ROS levels than control group. We conclude that exogenous quercetin is beneficial for nuclear maturation during porcine IVM and subsequent embryo development, most likely by increasing intracellular GSH synthesis, reducing ROS levels.

S5-3

Tet-controlled transgene expression in large animal models

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Increasing relevance of large animal models for biomedical research demands the development of sophisticated transgenic tools. Gene induction in a time-specific manner is a prerequisite for transgenes which interfere with the development of the animals or where a defined start of transgene expression is desired. Using the well-characterized Tet-On system, we have established transgenic pig models expressing either the synthetic CTLA4-Ig or a soluble variant of RANKL for immunodeficiency or osteoporosis studies.

Initially, we produced five independent founders carrying the transactivator under the control of the ubiquitous CAG promoter (CAG-TA) and chose lines with proper transgene expression for the addition of the controlled CTLA4-Ig or RANKL genes. Pigs were produced by nuclear transfer of genetically modified primary pig cells and subsequent transfer of the resulting embryos to synchronized gilts. Delivered piglets were analysed for the transgene status and double-transgenic pigs were stimulated by oral Doxycycline administration at an age of 8-12 weeks. In one founder with CAG-TA and a regulated CTLA4-Ig gene, significant amounts of the protein were detected after stimulation in blood serum, which inhibited T-cell activation in an in-vitro experiment. In another founder carrying CAG-TA and the regulated RANKL gene, the respective protein as well as increased levels of the osteoclast marker cathepsin K were detected in the blood. Without stimulation, no protein was detected in both cases.

Thus, here we present for the first time the establishment of gene-induction in pigs and prove the biological function of the regulated transgenes.

S5-4

Oxamflatin improves in developmental competence of somatic cell nuclear transfer porcine embryos

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Productive offspring from somatic cell nuclear transfer (SCNT) is the goal of most cloning laboratories. It has been demonstrated that the developmental competence of SCNT embryos in several species were significantly enhanced via treatment of histone deacetylase inhibitors (HDACi) such as trichostatin A (TSA) or scriptaid to increase histone acetylation. Here we report that 0.1 μ M oxamflatin for 6 h after activation improved the developmental competence of porcine SCNT embryos in vitro. Therefore, we optimized the application of another HDACi, oxamflatin for development of porcine SCNT embryos. We found that treatment with 0.1 μ M oxamflatin significantly enhanced the development SCNT embryos to the blastocyst stage. Oxamflatin increased the overall SCNT efficiency from 8.5 \pm 4.1 % (untreated group) to 28.9 \pm 6.3 % and increased number of cells (51.2 \pm 4.5 vs. 70.4 \pm 8.7) than that of the untreated embryos. Moreover, treatment of SCNT embryos with oxamflatin exert a dramatic amount of genetic control over pluripotency including Oct4, Nanog and Cdx2, the imprinting genes Igf2 and Igf2r, and the histone deacetyltransferase gene Hdac2.

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S5-5

LIF-Dependent, pluripotent stem cells established from inner cell mass of porcine embryos
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The pig is important for agriculture and as an animal model in human and veterinary medicine, yet, despite over 20 years of effort, there has been a failure to generate pluripotent stem cells analogous to those derived from mouse embryos. Here we report the production of LIF-dependent, so called naïve type, pluripotent stem cells from the inner cell mass (ICM) of porcine blastocysts by up-regulating the expression of KLF4 and POU5F1. The alkaline phosphatase-positive colonies resulting from re-programming resemble mouse embryonic stem cells in colony morphology, cell cycle interval, transcriptome profile, and expression of pluripotent markers, such as POU5F1, SOX2 and surface markers such as SSEA1. They are dependent on LIF signaling for maintenance of pluripotency, can be cultured over extended passage, and have the ability to form teratomas. These cells derived from the ICM of pig blastocysts are clearly distinct from the FGF2-dependent “primed” induced pluripotent stem cells described recently from porcine mesenchymal cells. The data are consistent with the hypothesis that the up-regulation of KLF4, as well as POU5F1, is required to create and stabilize the naïve pluripotent state and may explain why the derivation of ESC from pigs and other ungulates has proved so difficult. Currently, efforts are underway to establish similar lines from earlier 4-8 cell stage porcine embryos by utilizing tetracycline-inducible, bicistronic lentiviral vectors carrying POU5F1 and KLF4 transgenes for doxycycline-mediated regulation of expression. Future objectives are to test the potential of these lines to give rise to germ-line chimeras and produce a live offspring by somatic cell nuclear transfer.

S5-6

Human insulin expression in intestinal K-cells of transgenic swine

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Type 1 diabetes mellitus is caused by destruction of pancreatic insulin-producing β -cells. The objective of this study was to develop transgenic minipigs that express human proinsulin (hINS) in alternative (non- β) glucose-responsive cells. Gut K-cells are endocrine cells that secrete glucose-dependent insulinotropic peptide (GIP) postprandially. To target K-cells for insulin production, male Yucatan minipig fetal fibroblasts were transfected with hINS driven by the swine proximal GIP promoter (ssGIP-hINS). Selected cells were used for somatic cell nuclear transfer. Cloned embryos (n=166) were transferred (ET) to a surrogate sow and 11 embryos were collected on day-35. Fibroblasts from two embryos (F4 and F7) expressing hINS were used for serial cloning and ET into 8 surrogates, (F4, n=5; F7, n=3). C-sections yielded one transgenic piglet from F4 cells (pig 4-1) and two from F7 cells (pigs 7-1 and 7-2). Plasma was collected from weaned pigs after fasting and over 2 h following a glucose-rich meal. Postprandial glucose excursion was reduced in transgenic pigs. Human C-peptide in pig 4-1 was two-fold above fasting levels (53.3pM at 60 min post-feeding versus 27.4pM). C-peptide in pigs 7-1 and 7-2 were less elevated (14.8pM and

10.2pM, respectively at 60 min post-feeding), but were twice fasting concentrations (5.2 and 6.2pM, respectively). Immunohistological analysis of duodenal samples from F4 fetuses from two surrogates revealed insulin immunoreactivity specifically co-localized with GIP in K-cells (gestation days 56 and 68; n=5 and n=3, respectively). β -cell inactivation in offspring from transgenic founders will reveal the effectiveness of transgenic insulin to maintain normal glucose homeostasis.

S5-7

ZFN-mediated Ppar- γ gene targeting in pigs

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Gene targeting in large animals remains to be a difficult process with low efficiency due to the lack of established ES cell lines and the extremely low frequency of homologous recombination in somatic cells. Recently, zinc finger nuclease (ZFN) technology has shown great potential in genome editing in several species. However it is still unclear whether this technology is applicable in large animals. Here, we have introduced an approach that combines ZFN with the somatic cell nuclear transfer (SCNT) method to produce gene targeted pigs with a defined mutation in peroxisome proliferator-activated receptor γ (Ppar- γ) gene. A pair of ZFN showed high activity in a parthenogenetically activated (PA) embryo microinjection assay was introduced into porcine fibroblasts by electroporation. Totally 5 among 119 cell clones screened have been proven to be Ppar- γ mutants. Cells in a positive cell clone carrying a mono-allelic insertion in the targeted site, predicted to result in premature termination of PPAR- γ translation, were used as nuclear donor for SCNT. As results, the predicted ZFN-induced mutation was found in 2 cloned piglets, and the mutation has really resulted in disruption of Ppar- γ gene expression as shown in Western blotting. Thus, we have provided a high efficient platform for generating gene-targeted transgenic pigs for translational medicine and drug development. To our knowledge, this is the first success of endogenous gene targeting in large animals using the ZFN system.

Author	Page	Author	Page
Ackerman	44, 51	Cao	40
Adamski	37	Carlson	39
Agca	64	Carlson	68
Aigner	52	Cassady	44, 51
Ajuwon	36	Cernea	70
Alexenko	72	Chang	73
Alloosh	30, 43, 43, 45, 53, 57, 62, 62	Chawla	30, 45, 50, 53
Anderson	44, 51	Chen, C	40
Ando	57	Chen, L	45
Anthony	17	Chen, Y	73
Archibald	17	Chia	41
Asadi	72	Chmelarova	39
Avritscher	48	Clark, G	47
Ayares	28, 44	Clark, S	50
Bähr	28, 63, 71	Clark-Deener	33
Baker	72	Clausing	59
Basile	43	Coghlan	45, 48
Beaton	28	Colman	54
Bhargava	65	Conrad	21, 46
Bhutani	45	Darfour-Oduro	30
Bionaz	26, 29, 35	Das	48
Blake	40	Davis	47
Blikslager	49	de Castro	68
Blutke	51	Dean	68
Böcker	71	deMarco	68
Bode	58	DePaolo	22
Bolund	17	Dilger	21, 46
Borne	45, 48	Dobbs	33
Bost	63	Dobrinski	18
Bouchard	29, 58, 65, 67	Donninger	47
Bouchez	59	Dymon	29
Boyd	53	Earl	59
Braun	51, 52	Egan	39
Brocksmith	29, 58	Ellegaard	58
Brown	29, 58, 64, 67	Elmore	21
Buchtová	55	Emery	68
Burkhardt	34	Escobar	53
Burton	21	Evans	64, 64
Butler	41	Ezashi	72
Buzzitta	43	Fahrenkrug	33, 39
Byrd	30, 45, 50, 53, 62	Fan	56, 73
Calcaterra	41, 68	Feinberg	26
		Fezert	31

Author	Page	Author	Page
Fisher	53	Inoko	57
Forster	58	Isaacson	41
Fredrickson	61	Ishino	65
Fujishiro	66	Isom	40, 72
Furukawa	65	Itter	59
Ganderup	18, 31, 32, 58, 59, 59, 60	Ivarson	21
Ganjam	64	Iwai	66
Ganu	32	Iyer	48
Garbe	33	Jacobi	22, 49
Garrow	32	Jang	70, 71
Gehlot	48	Jensen	29, 33, 34
Gerrard	53	Jiyei	71
Gibbs	59	Johanning	68
Giroux	41	Johnson	21, 46
Glerup	19, 59, 60	Jonszta	55
Goldwasser	50	Kalita	48
Golightly	58	Kaneko	57
Gollin	67	Kang	70, 71
Gordon	47	Kaplan	68
Granada	54, 66	Kasten	53
Graw	51	Kawata	57
Green	35, 39	Kelso	41
Guo	45	Kessler	28, 34, 51, 52, 64, 71
Hackett	33	Kieffer	72
Hamazato	57	Kim, D	29
Hanazono	65	Kim, H	21
Hanks	29, 67	Kim, K	33
Haschek	61	Kim, S	70, 71
Hayashi	31, 32, 60	Kitagawa	57
Henson	67	Klymiuk	28, 31, 34, 51, 52, 63, 71
Herbach	51, 71	Kobayashi	21, 66
Herring	19	Kong	65
Hiemstra	29, 58, 67	Koo	71
Hollister	26	Krueger	54, 54, 66
Honda	55	Kurome	28, 34, 51, 52, 63, 71
Hrncir	38	Kusewitt	45
Hsiao	61	Kwon	70, 71
Hu	45	Kyllar	55
Hudson	63	Lai	56, 73
Hulet	20	Landbrecht-Schessl	52
Hurley	29, 35	Larsen	68
Hwang	48	Laughlin	27
Imaeda	57	Lee, B	70, 71

Author	Page	Author	Page
Lee, K	21	Noriega	68
Lei	49, 61	Odle	22, 49, 63
Lewin	40	O'Gorman	35, 36
Lewis	67	Ohshima	57
Li, C	35	Ouyang	56, 73
Li, F	73	Paris	51
Li, N	45	Park, H	71
Li, S	56	Park, S	70, 71
Li, W	56, 73	Pei	73
Li, X	56	Piedrahita	23
Lima	35	Pierson	23
Lin	22	Pieters	52
Liu, J	29, 58, 67	Piller	63
Liu, Z	56, 73	Pipinos	68
Lu, H	36	Pohle	62
Lu, Y	69	Portis	45
Ma	30	Potu	36
Maehara	66	Prather	27, 28, 35, 36, 39, 40, 41, 67, 72, 72
Maki	29, 50	Pratt	43
Makin	60	Prehn	37
Mansharmani	25	Price	53
Mather	30	Puk	51
Matsumoto	66	Putnová	55
Matsunari	55, 66	Radic	71
McCall	68	Reecy	23
McKenney	45, 50, 53	Reed	54, 54, 66
Medin	67	Reinholt	53
Merritt	62	Renner	37, 51, 52
Mhedhbi	59	Reyon	33
Miller	61	Roberson	53
Mirando	22	Roberts	72
Mizukami	65	Robson	24
Moeser	49	Roca	32
Monaco	26, 29, 35	Rogers	47
Moon	70, 71	Rohrer	37
Motsinger-Reif	44	Rohret, F	47
Nagashima	28, 34, 51, 52, 55, 63, 66, 71	Rohret, J	47
Nakano	55, 66	Römisch-Margl	37
Navratil	31, 60	Roscher	37
Nguyen	51	Ross, J	68
Nicholls	67	Ross, M	67
Noel	68	Rudenko	25
Nonneman	37		

Author	Page	Author	Page
Rund	33, 34, 47	Tamuli	48
Samuel	27, 36, 41, 72	Tan	39
Sarmah	48	Tellez	54, 66
Sato	66	Telugu	72
Schachtschneider	38, 41, 52	Teson	39
Scheffler, J	53	Tian	56, 73
Scheffler, T	53	Tinkey	48
Schmidt	47	Trebichavsky	39
Schook	30, 32, 33, 34, 38, 41, 47, 52	Trickey	64
Schuleri	25	Uhl	69
Schultz	45, 53	Umeyama	55, 66
Schwinzer	71	Unterreiner	67
Seissler	63	Uthamanthil	45, 48
Shanmuganayagam	54, 54, 66	van Bürck	S4-1
Shih	36	van der Laan	58
Sinha	72	VanEyck	53
Skydsgaard	60	Varghese	66
Skytte	60	Velandar	41, 68
Smiltneek	54	Voytas	33
Song	56, 73	Vukamanic	68
Sparks	45, 62	Wallner	34
Spate	72	Walters	27, 28, 36, 40, 41, 64
Spencer	30, 50	Wang	40
Splichal	38, 39	Wang	68
Splichalova	38, 39	Wang, C	56
Spollen	40	Wang, J	45
Spretz	68	Wang, Q	45
Stafford	48	Wang, X	47
Stahl	63	Wanke	51
Stehlík	55	Ward	36
Stein	41	Watanabe	55, 66
Stembírek	55	Webber	72
Stice	25, 69	Weichmann	54
Streckel	52	Wells	26, 27, 28, 35, 36, 39, 70
Struzynski	47	West	25, 40, 69
Sturek	30, 36, 43, 43, 45, 50, 53, 57, 62, 62	Wheeler	26, 29, 35, 50
Suhre	37	White	38, 41
Sutovsky, M	36	Whitworth	27, 39
Sutovsky, P	36	Whyte	27, 40, 72
Swanson	33, 34	Wiedmann	37
Takeuchi	55	Wolf	28, 31, 34, 37, 51, 52, S4-1, 71
		Woodard	50

Author	Page	Author	Page
Wright	48		
Wünsch	31, 34, 51, 52, 63, 71		
Xi	49		
Xiao	40		
Yang, D	56, 73		
Yang, F	41		
Yang, H	73		
Yang, J	69		
Yang, S	45		
Yeoman	38, 41		
Yokoo	55, 66		
Yokote	66		
Yu	40		
Zang	33		
Zhang	73		
Zhao, B	56, 73		
Zhao, J	27, 36, 41		
Zhao, Y	56, 73		
Zhong	40		