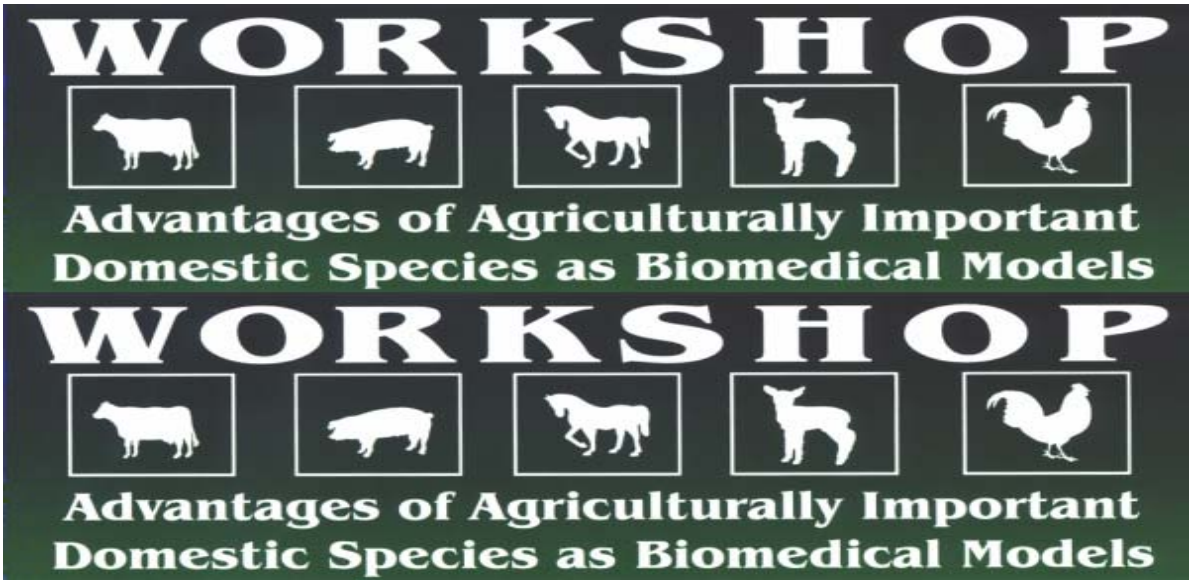

Speaker Abstracts:

Heart-Healthy Pork, and Green Eggs and Ham: Genetically Modified Pigs for Medicine and Agriculture

Randall S. Prather, Yanhong Hao, Rongfeng Li, Jason W. Ross, and Jeffrey Whyte, Division of Animal Sciences, and the National Swine Resource and Research Center. University of Missouri, Columbia, Missouri

Pigs are an increasingly important model for studying and understanding human medicine. The National Institutes of Health spends over $100M per year extramurally on pigs. These funds support studies in cardiovascular disease, atherosclerosis, cutaneous pharmacology, wound repair, dermatology, cancer, diabetes, ophthalmology and toxicology research, lipoprotein metabolism, pathobiology of intestinal transport, injury and tissue repair as well as for developing the pig as an organ source for xenotransplantation. Pigs are popular because they are readily available, their research model phenotype in many cases is more similar to humans than rodents are capable of providing, their physiology is more similar to humans than rodents, their physical size is more similar to humans which provides adequate amounts of tissue for analyses such as proteomics. The ability to make genetic modifications in the pig has dramatically expanded their usefulness as research models. A modification as simple as adding the enhanced green fluorescent protein to the pig has produced tissues being utilized for research on repairing damaged retinas, isolation of blood-derived adult stem cells, umbilical stem cells, adipocyte stem cells, cardiac stem cells, wound healing, and spermatogonial cell transplantation. Other genetic additions or knockouts have resulted in pigs that have been used for xenotransplantation, sources

of pharmaceuticals (pig mammary gland is the only known livestock tissue that can properly post-translationally modify the Vitamin K-dependent proteins involved with hemostasis), models of retinitis pigmentosa (pig eyes are of similar size and physiology to human eyes), cardiovascular disease (plaque development is almost identical to that observed in humans), Huntington's disease, as well as improvements in the meat quality and efficiency of production animals. While a variety of techniques can be used to add genes, only homologous recombination followed by nuclear transfer has been used to create knockout models. The porcine genomic code will prove to be a valuable tool adding to the agricultural and biomedical usefulness of the pig. The swine genome sequencing project is underway, and for research purposes we, in collaboration with the University of Illinois, are cloning the pig whose genome is being sequenced.

PLCζ (zeta), the Sperm Factor of Mice, Bovine and Men

Raphael Fissore, Department of Veterinary & Animal Sciences, University of Massachusetts, Amherst, Massachusetts

My laboratory's interests lie in the elucidation of the signaling mechanisms that underlie fertilization and the initiation of development in mammals. It is now well known that the universal mechanism employed by the sperm to induce egg activation, i.e., the exit of the meiotic program and the commencement of mitotic divisions, is an increase in the intracellular concentrations of free calcium ($[Ca^{2+}]_i$). In mammalian eggs, fertilization induces a characteristic series of periodical $[Ca^{2+}]_i$ rises that last in excess of 4 hours and that are called $[Ca^{2+}]_i$ oscillations. My laboratory's focus has been on identifying the sperm molecule(s), the sperm factor, responsible for inducing $[Ca^{2+}]_i$ oscillations in bovine, human and mouse eggs.

For nearly 30 years, the prevailing view was that the sperm engaged a receptor in the egg surface whereby production of IP_3, the ligand that ultimately induces $[Ca^{2+}]_i$ release, was initiated. A paradigm shift occurred when it was demonstrated that injection of sperm extracts, all of our studies used porcine sperm, induced fertilization-like oscillations across eggs of all mammalian species. Ten years after this initial observation, phospholipase Cζ (zeta; PLCζ), the putative sperm factor that belongs to a family of enzymes responsible for the production of IP_3, was identified in mouse testis. Significantly, most of the key preliminary biochemical studies that cemented the sperm factor hypothesis used porcine sperm, given the abundance of the material provided by this species and the established conservation of the pathway under consideration.

The identification of PLCζ as the sperm factor offers several important applications for agriculture and biomedicine: a) injection of PLCζ mRNA has already been shown to be an excellent method of parthenogenetic egg activation, which will improve the success of nuclear transfer procedures and will facilitate the generation of parthenogenetic stem cells lines in all species; b) in light of PLCζ's sperm-specific expression and its high sequence homology from chickens to humans, tests that rely on its immuno-detection could be used as a marker of male fertility; c) given that nearly 3% of couples that undergo the intracytoplasmic sperm injection (ICSI) procedure fail conceive and that most of these failures are due to lack of egg activation, PLCζ mRNA or its recombinant protein could be developed as therapeutic tools.

In spite of PLCζ's fundamental role in egg activation, the regulation of its function and whether its malfunction undermines ICSI success remains to be determined. To answer these questions, domestic species may serve as better models than rodents. For instance, ICSI in large domestics results in very low embryo development and initial observations show that in these species ICSI fails to induce $[Ca^{2+}]_i$ oscillations, which insinuates problems in the delivery and/or activation of PLCζ. Importantly, despite the high degree of homology, the linker region of PLCζ is poorly conserved, and this sequence variation is magnified between bovine/porcine and human isoforms. Given that the activity of PLCζ exhibits varying degree of species-specificity, i.e., higher activity in eggs of homologous rather than heterologous species, faster progress toward the understanding of molecular determinants of activity will be achieved by undertaking studies that use gametes from a wide spectrum of species.

Studies designed to elucidate the signaling pathways involved in mammalian fertilization are a perfect target for joint funding by NIH and USDA. The underlying molecular mechanisms in both egg and sperm are highly conserved across species and, besides offering greater availability of gametes, the temporal requirements of oocyte growth and maturation, fertilization, early embryonic gene expression and pre-implantation embryo development in domestic species are much closer to those exhibited by humans than those displayed by rodents.

Domestic Rabbits and Cattle as Models for Cardiovascular Diseases and Therapeutic Cloning

X **Cindy Tian, Department of Animal Science, University of Connecticut, Storrs, Connecticut**

The domestic rabbits as models for research on cardiovascular diseases. Cardiovascular disease (CVD) is the No. 1 killer in America, costing 1 in every 2.6 deaths and $393.5 billion annually for treatments in the US alone. It is unethical to create CVD in humans or to test treatments/conduct mechanistic studies directly on CVD patients. Although studies of the cardiovascular system has benefited significantly from the use of transgenic mouse models, rodents are naturally resistant to the development of atherosclerosis, which is the cause of heart attack in humans. Rabbits, however, do develop diet-induced atherosclerosis because of their similarities to human cardiac physiology in numerous aspects: 1) as in humans, adult rabbits express the β-MHC while the mouse only expresses the isoform; 2) rabbit lipoprotein metabolism is very similar to that of humans (LDL-mammals), but very different from mice which are HDL-mammals; 3) cholesteryl ester transfer protein (CETP), which plays a central role in the atherosclerotic process, is abundant in both human and rabbit plasma but absent in the mouse. Additionally, rabbits have a short gestation length (30-31 days), are very prolific (4-10 babies/litter), relatively inexpensive, and easy to handle compared to pigs or Rhesus monkeys. Furthermore, technology/equipment developed for human infants, for both invasive and noninvasive cardiovascular studies, could be used with no modifications in adult rabbits which are similar in weight and size to human infants. All of these unique characteristics make the rabbits ideal models for human CVD. However, since the early 1980s, the majority of CVD research was conducted in the mouse, not in rabbits. Despite of more than 15 years of intensive research, the best mouse model created still do not develop human-like CVD. How did a less suitable model become the mainstream animal of choice in CVD studies? This is because it was not possible to manipulate the rabbit genome by knockout/knock-in (KO) technology to make them even more "human-like". Today, with the development of technologies of homologous recombination in somatic cells and nuclear transfer, creating more "human-like" rabbits by modifying their genome is a reality. The joint funding between NIH and USDA is recommended to improve the efficiency of nuclear transfer in rabbits and to create a family of KO model rabbits for CVD research.

Domestic cattle as models for human therapeutic cloning. Human therapeutic cloning, in which a patient's somatic cells are reprogrammed by the nuclear transfer (NT) technology for embryonic stem cells (ESC) generation and targeted differentiation, has tremendous potential for tissue regeneration and replacement. Its development, however, is greatly limited by the lack of human oocytes and controversies surrounding the generation and destruction of cloned human embryos. Interspecies nuclear transfer (iNT), such as those between non-human primate/human (somatic donor cells) and a species with sufficiently accessible oocytes such as the cattle or rabbit (recipient oocytes), provides a good model to optimize the human therapeutic cloning technology. The choice of cattle is also important because bovine early embryo development closely mimics that of humans and the nuclear reprogramming time (~ 6-7 days), during which the differentiated somatic cells become totipotent, is similar between humans and cattle, whereas in the mouse, the reprogramming time is only 2 days which is too short to model humans. The joint funding between NIH and USDA is recommended to improve the efficiency of interspecies nuclear transfer between cattle and non-human primates, to trace the fate of donor/recipient mitochondria, to study the compatibility of reprogramming factors and to generate ES cells from such interspecies NTs.

Developmental Programming of Adult Disease: Understanding the Impact on Human Health and Animal Production,

Larry Reynolds, Center for Nutrition and Pregnancy, North Dakota State University, Fargo, ND

'Fetal Programming' (or, more correctly, 'Developmental Programming') refers to the concept, based originally on epidemiological observations in humans but now corroborated with controlled studies in several animal models, that insults to the fetus or neonate have health consequences not only during the immediate postnatal period but, in fact, throughout adulthood (*see* Barker, D.J. Developmental origins of well being, Phil Trans Royal Soc, London 359:1359-1366, 2004). The insults include things such as poor maternal nutrition during pregnancy, and the health consequences include highly increased odds of cardiovascular disease or metabolic syndrome. In livestock, compromised pregnancy (as reflected by fetal growth restriction) often occurs in normal production settings, and exhibits many of the consequences seen in humans, including poor postnatal growth and body composition, and dysfunction of specific organs, thereby resulting in poor animal performance (*see* Wu et al., J Anim Sci 84:2316-2337, 2006). Thus, animal models provide an important tool to investigate the mechanisms responsible for developmental programming in humans and livestock (*see* Armitage et al., J Physiol 561:355-377, 2004). In this regard, domestic sheep make an outstanding animal model because of their similar physiology and endocrinology to that of other mammals, their extended gestation length, the ability to examine multiple stages of pregnancy, the large amounts of tissue available for study, the ability to chronically instrument the mother and fetus, and the large body of basic and production data in this species. Moreover, several models of compromised pregnancy in sheep show striking similarities with compromised pregnancy in humans. Our past and current NIH and USDA-NRI funding has enabled us to develop and characterize, along with our collaborators, several models of compromised pregnancy in sheep, including those due to maternal nutrition, maternal age, maternal and fetal genotype, multiple fetuses, and various interactions among these. Our studies using these models have led us to conclude that compromised placental growth and vascular development/function are primary contributors to fetal growth restriction (*see* Palmieri et al., Placenta, Article in Press, oi:10.1016/j.placenta.2006.08.003, 2006; Reynolds et al., Placenta 26:689-708, 2005; and Reynolds et al., J Physiol 565.1:43-58, 2005). In addition, we have proposed that relatively simple pharmacological or nutritional treatments that are designed to alleviate the compromised placental growth and vascular development/function can be used to 'rescue' fetal growth in these compromised pregnancies (*see* Reynolds et al., J Physiol 572:51-58, 2006). Thus, we believe that animal models will continue to contribute to our understanding not only of the mechanisms responsible for compromised pregnancies but also their management. We also believe that the sheep provides a powerful model animal for studies related to developmental programming, and which have profound implications not only for animal production but also human health.

Cardiac Vulnerability Models in Fetal Sheep

Kent L. Thornburg, Director, Heart Research Center, Oregon Health & Science University, Portland, Oregon

Area of Research. The Heart Research Center studies cardiovascular "programming." Programming is the biological response to environmental stressors in the womb that lead to vulnerability to disease in adult life. We study many models that affect the heart and blood vessels. For example, embolization of the placenta leads to fetal hypoxemia and nutrient deprivation during which the heart stops growing and maturation of cardiomyocytes ceases. Our anemia model remodels the coronary tree for life.

Relevance to Animal Agriculture and Biomedicine. The roots of vulnerability for cardiovascular disease lie in prenatal life for both humans and animals. Understanding the roles of oxygen, nutrients and chemical signals in establishing vulnerability of the cardiovascular system applies to animals that are grown for agriculture as well as humans. Our models of anemia, placental insufficiency, volume and pressure overload, cortisol and thyroid excess are all relevant to the cardiac health of both domestic animal species and humans.

Advantages of Domestic Species as Translational Models for Biomedicine. The sheep model is the most widely used model for fetal cardiac development world wide for several reasons: 1) Sheep fetuses tolerate chronic catheterization extremely well. Indwelling catheters can be maintained for months. 2) The fetal heart is about the same size as the human fetal heart near term. The heart can be studied by implanting ultrasonic crystals, high fidelity pressure transducers, EKG leads etc. 3) Fetal models mimic human diseases and lend themselves to translational studies. Fetal placental embolization yields a fetus with a physiology similar to one type of growth retarded human baby. Reversal therapies can be studied in such models.

Advantages of Domestic Species over Human Studies. Determining the basis for human disease cannot be determined without animal models because the biology is too complex and repeated access to the human fetus is not feasible. Imaging methods are not adequate to make high resolution measurements long term.

Focused Areas of Research Recommended for Joint Funding between NIH and USDA: 1) Defining the Genome. Multiple array chips are needed. 2) Reversing the effects of programming. Nutritional deficit leads to poor kidney growth, inadequate pancreatic development, upregulation of pro-oxidant systems, abnormal cardiomyocyte endowment, and an abnormal coronary tree. Models in domestic species could take the lead in specific programs that discover and reverse detrimental effects of nutrition, hypoxemic and corticosteroid stress in animals. Discovery methods would include DNA microarray analysis, plasma proteomics, and physiological markers. Therapy would include gene therapy, stem cell therapy, nutrient therapy, pharmacotherapy, anti-epigenesis, RNA therapy and physiological manipulation. New imaging methods can be used to follow some therapies. 3) Well designed long term studies on programming. In order to study the effects of stressors in utero, animals need to be followed for some 3-5 years in sheep. Study sections are reluctant to support these studies because of the time frame of a funding period. Because programming is clearly linked with ageing, an ageing model in a domestic species would be very valuable.

Developmental Programming of Reproductive and Metabolic Health and Disease

Vasantha Padmanabhan, Director of Pediatrics, Department of Pediatrics, Obstetrics and Gynecology and Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109-0404, USA

The inappropriate programming of the reproductive system by fetal exposure to excess steroid hormones is of concern, especially in the female. At risk is the female fetus whose mother has been exposed to exogenous steroids for a variety of reasons: failed contraception and continued exposure to contraceptive steroids, use of anabolic steroids or inadvertent exposure to environmental compounds with estrogenic or androgenic activity. The sheep is exceptionally well suited for investigating fetal origin of adult reproductive and metabolic disorders. The developmental time line of sheep (5 months gestation and 7-month interval from birth to puberty) is ideally suited for conducting sequential series of integrated studies of the progression of metabolic/reproductive disruption from the developmental insult to manifestation of adult consequences. Major benefits of using sheep include knowledge of established critical periods to target adult metabolic and reproductive defects, a rich understanding of reproductive neuroendocrine regulation and ovarian differentiation that parallel humans, availability of non-invasive approaches to sequentially monitor follicular dynamics as well as established surgical approaches to obtain hypophyseal portal blood for measurement of hypothalamic hormones, and the ability to perform studies in natural setting keeping behavioral interactions intact. Of even greater importance is the ability to chronically instrument fetus and mother for determining early endocrine perturbations. Studies of others and ours using the sheep as the animal model reveal that prenatal exposure of the female to excess testosterone (T) leads to an array of adult reproductive disorders that include LH excess, functional hyperandrogenism, neuroendocrine defects, multifollicular ovarian morphology, and corpus luteum dysfunction culminating in early reproductive failure. From a neuroendocrine perspective all 3 feedback systems estradiol negative, estradiol positive and progesterone negative feedback are compromised. At the ovarian level, multifollicular morphology stems in part from persistence of follicles as well as enhanced follicular recruitment. These reproductive defects lead to progressive loss of cyclicity. Prenatal T treatment also leads to fetal growth retardation, an early marker of adult reproductive/metabolic diseases, insulin resistance, hypertension and behavioral deficits. Collectively, the reproductive and metabolic deficits manifest in the prenatally T-treated sheep highlight the usefulness of this species as a model in understanding the developmental origin of human fertility disorders such as polycystic ovarian disease, congenital adrenal hyperplasia, premature ovarian failure and metabolic syndrome.

The Neonatal Pig as a Dual-Use Model for Studies of the Developmental Regulation of Protein Metabolism

Teresa A. Davis, Marta L. Fiorotto, and Douglas L. Burrin
USDA/ARS Children's Nutrition Research Center,
Baylor College of Medicine, Houston, Texas

The neonatal period is a stage of rapid growth in which a number of environmental factors, particularly nutrition, have a significant impact. Dietary protein is utilized very efficiently for the deposition of body protein in the healthy neonate because they are capable of a greater increase in protein synthesis in response to feeding than at later stages of development. This is most pronounced in skeletal muscle, the fastest growing and largest protein mass in the body. Our studies in neonatal pigs have focused on identifying the mechanisms that enable neonates to maximally utilize dietary protein for growth. We have shown that the feeding-induced stimulation of muscle protein synthesis is independently mediated by an enhanced sensitivity to the post-prandial rise in insulin and amino acids. These effects are modulated by positive and negative regulators of the insulin and nutrient signaling pathways that lead to translation initiation and the translation initiation factors that regulate mRNA binding to the ribosomal complex. Leucine acts as a nutrient signal to rapidly stimulate translation initiation but requires that other amino acids be supplied to sustain the effect. Catabolic conditions such as inflammation decrease muscle protein synthesis by reducing the activation of translation initiation signaling. Our studies to identify the mechanisms that regulate the high rate of neonatal muscle growth have led us to expand our studies to examine the mechanisms by which growth promoting agents like somatotropin enhance muscle mass.

Although the purpose may differ, the objective of promoting muscle mass is important both for animal agriculture and human health. A primary goal of animal production is to increase growth, muscling, and feed efficiency. Improved understanding of the regulation of protein metabolism at the basic level will lead to improved production performance and enhanced efficiency of protein utilization in swine nutrition. Identification of the mechanisms that regulate protein deposition in neonates is enabling the development of new strategies to optimize the nutritional management of low birth weight infants. Because muscle mass and function are intimately associated, strategies to accelerate muscle growth promote functional development and, thus, the overall outcome is improved. In patients across all ages, loss of muscle mass occurs readily with many chronic diseases and is associated with increased morbidity and mortality. The ability to accelerate muscle protein accretion and restore muscle mass is an important variable in the clinical outcome in these conditions. Because the process of muscle regeneration recapitulates ontogenic changes, the results from developmental studies on the regulation of muscle anabolism also are pertinent to conditions in which muscle mass is compromised in the mature individual. Thus, understanding the regulation of muscle growth has implications for improving both animal agriculture and human health.

The pig is a unique model to study the impact of nutrition and disease on growth and functional development of infants. The anatomy, physiology, body composition (except fat), and intermediary metabolism are generally similar in the infant and piglet. The large body size of the piglet also permits the use of a range of experimental approaches that are not feasible in the neonatal rodent such as repeated sampling, whole body tracer kinetics, organ substrate flux, and tissue blood flow. Importantly, the mode of nutritional support, via the parenteral or enteral route, can be examined along with the level of intake and composition of nutrients. Unlike some domestic species, large litters of the pig allow multiple dietary treatments. Immunological immaturity can be an advantage to the design of some studies. Limitations of the swine model include inadequate availability of species-species reagents such as antibodies and sequence

information and the difficulty in generating transgenic models. Long-term studies can be limited by large adult body size.

For a number of reasons, studies in the piglet are more informative than direct studies in the human infant for assessing the regulation of nutrient metabolism in human health and disease. Use of the piglet overcomes the ethical limitations of conducting research in human infants including tissue sampling, surgical manipulation, and administration of radioactive isotopes and experimental drugs. The piglet also offers a unique model organism in which to study the effects of nutrition on the etiology and pathogenesis of major diseases, including infections and gastrointestinal pathology that incur with prolonged hospitalization of young infants. Because of the faster rate of growth in the piglet, the power to detect the impact of dietary treatment is increased. Gene therapy approaches can also be evaluated in the pig model.

Important areas of research that would use the fetal and neonatal piglet as an experimental model and that would benefit from joint funding by USDA and NIH include studies to develop strategies, including nutritional, pharmacological, or gene therapies, to improve the growth and functional development of low birth weight and premature infants who are otherwise healthy. Studies to identify the role of nutrition on the etiology, pathogenesis, and prevention of major diseases incurred in preterm and term neonatal infants are also of importance. In the agricultural setting, the newborn piglet is vulnerable to similar problems (fetal growth retardation, respiratory infections, diarrhea, etc.), and thus strategies to reduce the incidence, severity, and duration of these conditions will be beneficial to both species. The technically complex aspects of studies in newborn (premature or term) piglets require biomedical facilities and conditions that are generally not available in agricultural research facilities. Long-term studies of the effects of early life nutrition on the development of adult disease are another important area of research that would benefit from joint collaboration and funding. As mechanisms are identified, interventions to mitigate the development of these diseases during adulthood need to be developed and tested. Such studies are logistically difficult and time-consuming, and would merit from collaborations between animal agriculture and biomedical researchers to provide the necessary resources.

From Bench to Bedside: Where Do Obese Pigs Fit In?

Michael E. Spurlock, Department of Human Nutrition and Animal Science, Iowa State University, Ames, Iowa

The natural pathogenesis of type II diabetes typically involves a genetic predisposition to obesity, chronic inflammation, and a gradually increasing impairment of insulin action in a "prediabetes" condition, which has also been termed the metabolic syndrome or cardiometabolic risk. In later stages there is an increase in fasting blood glucose that best defines diabetes. The prevalence of obesity and the metabolic syndrome in the U.S. population is increasing at an alarming rate. Furthermore, the economic burden of diabetes, a common outcome of the metabolic syndrome, is estimated at some $100 billion annually, with the majority of this relating to Type II diabetes. Thus, obesity and the metabolic syndrome are of unequivocal importance to society, as are the losses in quality of life and shorter life expectancies which accompany these diseases. Despite aggressive research to define the molecular links between obesity, chronic inflammation, and insulin resistance, the underlying biochemical mechanisms have not been clearly identified. Rodent models are commonly used by obesity and diabetes researchers because they are inexpensive to maintain, have a sequenced genome, and are easily modified by genetic engineering. However, apart from the Zucker rat, which is extremely hyperphagic due to the absence of a functional leptin receptor, rodent models which develop 3 or more of the clustered risk factors required for a consensus declaration of the metabolic syndrome are lacking, and other animal models showing consistently 4 or more risk factors have not been identified. Furthermore, there are clear metabolic and physiological differences between humans and rodents, and these differences have undoubtedly slowed progress and complicated the translation of biomedical research findings into effective preventive or intervention therapies for obesity and its co-morbidities.

The pig is an attractive biomedical model for energy metabolism and obesity in humans because it is devoid of brown fat, postnatally, and because of its similar metabolic features, cardiovascular system, and proportional organ sizes. Furthermore, adipose depots in pigs are of sufficient size that multiple assays can be carried out on adipocytes or stromal vascular cells without pooling across depots or animals. This is advantageous for defining the molecular basis of regional differences in adipocyte function. Furthermore, the consensus opinion among researchers is that the pig develops atherosclerotic lesions much more similar to humans than do rodents, and is in fact a better restenosis model altogether. As regards obesity and insulin resistance, the Ossabaw breed of minipig has particular appeal. These pigs were deposited on Ossabaw Island, Georgia, U.S.A. in the 1500s by Spanish explorers, and since then the ocean has remained an impenetrable barrier to emigration to the mainland. Ossabaw miniature swine may recapitulate the natural pathogenesis of type 2 diabetes due to their "thrifty genotype" that enabled survival in the feast and famine ecology of Ossabaw Island. Ossabaw swine develop 5 of the 6 characteristics of the metabolic syndrome, with significant central obesity, high blood pressure, primary insulin resistance, dyslipidemia characterized by a marked hyper-triglyceridemia and increased LDL:HDL, and also significant evidence of coronary artery disease. This talk will focus on these findings and will emphasize the considerable potential value of the Ossabaw minipig to the scientific community.

Nutritional Biochemistry of the Developing Neonate: Insights Gleaned from a Piglet Model

Jack Odle, Department of Animal Science, North Carolina State University, Raleigh, North Carolina

Neonatal animals from all mammalian species face a number of biological stressors following birth that result in high rates of postnatal morbidity and mortality. For example, the U.S. ranks 28th in the world with respect to neonatal mortality, with rates among infants weighing less than 1.5 kg approaching 40%. Similarly, reproductive inefficiencies, including low postnatal survival, have been long recognized as major obstacles facing production agriculture. Recent estimates from the USDA suggest that up to 15% of piglets born alive do not survive through weaning age. In both cases, neonates that are born prematurely or small-for-gestational-age are at greater risk. These national statistics underscore the need for research to increase our understanding of the various stressors that impinge upon the neonate in order to minimize their deleterious effects. Development of these research and management strategies constitutes a formidable challenge to both medical and agricultural sciences. Recognizing the commonality of the underlying biology, my laboratory has worked to develop the neonatal piglet as a pediatric model species, with a commitment to address at a fundamental level problems that are relevant to both agricultural and biomedical sciences.

In many ways, the neonatal piglet is an ideal pediatric model for studies of nutrition and metabolism as well as gastroenterology. As a domestic livestock species, there is less public aversion to the use of terminal or invasive procedures than exists for non-human primates or young canines. Most of the 50+ land-grant universities have production farms that can supply animals of reasonable uniformity at an affordable price. Compared with rodent pups, piglet gastrointestinal physiology and function more similar to humans. Weighing about 1-2 kg at birth, piglets are of a size that allows both Areduction science@ investigations of tissue/cellular metabolism, but also are large enough for catheterization and serial blood sampling beyond what a small rodent pup can afford. In addition, the comparatively rapid growth rates of piglets also make them a sensitive model for testing dietary variables within a short time period. Thus, the piglet model allows a full range of in vitro preparations for screening experiments as well as whole-animal preclinical studies prior to extending efforts directly to infants. For these reasons, the human infant formula companies have relied heavily on the piglet model for nutritional studies.

Primary efforts of my laboratory have focused on the ontogeny of lipid metabolism as well as intestinal growth and development during the early postnatal period. While findings from this program relate directly to agriculture, they also have important ramifications for human health. Priority areas of active investigation include:

(1) Safety and efficacy of supplemental long-chain polyunsaturated fatty acid (PUFA) for suckling infants. Using the piglet model, we provided seminal data leading to FDA approval of arachidonate and docosahexaenoate supplementation to infant formulas in 2002.

(2) Childhood obesity and neonatal metabolic imprinting B does early nutrition influence lipid metabolism later in life? We are using the anti-adipogenic effects of conjugated linoleic acid to examine determinants of lipid accretion in the suckling neonate.

(3) Optimization of nutritional support to enhance intestinal health and repair following gastroenteritis. Stimulative effect of PUFA, epidermal growth factor, arginine and plasma protein have been demonstrated. We also have examined effects of IGF and alanyl-glutamine.

(4) Investigation of factors that enhance the utilization of medium-chain triglycerides, including effects of fatty acid chain length, emulsification, and supplemental carnitine.

(5) Delineation of the biochemical limitations in fatty acid oxidation in the low birth-weight neonate.

We have shown enhanced peroxisomal oxidation and diminished ketogenesis in suckling piglets. These accomplishments (among others) illustrate the synergy that can result from the appropriate application an *agriculturally important domestic species as a biomedical model*. While no animal model is perfect, it is abundantly clear that fundamental comparative studies illuminate common biological motifs as well as unique idiosyncrasies among species. This approach will propel basic science forward and will under gird practical applications in agricultural, biotechnology and biomedical sciences as a result. Some limitations to programmatic advances in this area include the historical segregation of agriculture schools from medical schools, lack of full genomic sequences for domestic livestock species (swine in particular) and the lack of federally-funded Centers and other umbrella organizations to bring agricultural and medical scientists together to facilitate scientific exchange. Opportunity exists to catalyze and enhance synergy between agriculture and medicine if federal granting programs at NIH, NSF and USDA would organize with this explicit purpose in mind.

Pathogen Emergence and Shifts in Infectious Disease Patterns: Studies in Real-Time Using Domestic Animals

Guy H. Palmer, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington

Of the >1,400 described microbial pathogens affecting humans, ~60% also infect animals; of the emerging human pathogens in the past 30 years, an even higher percentage have animal reservoirs or have "crossed" species to allow direct human to human spread. Spongiform encephalopathies, SARS virus, and Asian H5N1 avian influenza represent the most dramatic public health concerns—however the same pattern is evident in numerous bacterial, viral, and parasitic pathogens. As expected there is wide diversity in the animal species that serve as the reservoirs for emerging human pathogens, although perhaps surprisingly, ungulate animals are disproportionately represented—likely attributable to the close contact between humans and livestock as opposed to rarer human-wildlife interaction. Emergence of new pathogens, accompanied by intrinsic uncertainty, has broad and deep impact on both the public health mission of NIH and the economic and food safety missions of USDA.

Emergence reflects endpoints of a continuous process of pathogen genetic change and fitness selection within the animal reservoir hosts—a process culminating in "jumps" signaled by major shifts in virulence, transmission efficiency, and host species. Understanding the frequency of these genetic jumps and the determinants of whether the new pathogen establishes and maintains itself in the host population represent major gaps in knowledge directly relevant to human and animal health. Domestic livestock represent the ideal host population to address this knowledge gap due to the direct role of livestock as disproportionately important reservoirs for emergent human pathogens and due to the ability to assess pathogen change under near real-time conditions through longitudinal sampling. The dynamic of pathogen genetic change and selection requires diversity in the host population, which is well-represented in livestock, and natural transmission events that provide selection.

Pathogen evolution in reservoir populations is understudied and not well represented among the priorities of the USDA-NRICGP. There are few livestock models developed to the point where they are competitive for NIH investigator initiated support and key gaps in our knowledge of pathogen emergence remain unaddressed. This coincident lack of USDA and NIH support has long-term consequences as new investigators capable of testing hypotheses regarding pathogen emergence in natural host populations are not being trained nor retained. Approaches to addressing this research need include a broadening of the investigator-initiated research mission in the NRICGP and/or a targeted USDA-NIH program to develop models of pathogen evolution in livestock.

Horse Sense and HIV: Using an Equine Model to Study Correlates of Lentivirus Immune Control

Robert H. Mealey, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington

A protective HIV-1 vaccine will undoubtedly need to induce cytotoxic T lymphocytes (CTL) to eliminate infected cells, but development of such a vaccine has been hampered because the correlates of CTL-mediated protection against lentiviruses are not known. In addition to CTL, complete protection against lentiviral infection will most likely require neutralizing antibody. However, vaccines that elicit antibodies that neutralize diverse HIV-1 isolates and result in consistent protection against challenge with heterologous pathogenic viruses have not been developed despite enormous effort. Some of the most significant knowledge gaps inhibiting progress towards the development of a protective HIV-1 vaccine are how to overcome viral escape from CTL and neutralizing antibody, and how to consistently induce these responses in an MHC diverse population.

Equine infectious anemia virus (EIAV) is a lentivirus that causes equine infectious anemia (EIA) in horses worldwide. Recurrent episodes of viremia and clinical disease occur, and each episode is associated with the emergence of an antigenically distinct EIAV variant as defined by neutralizing antibody. Although infection can be severe and fatal, clinical episodes usually become less severe and most horses eventually control the infection, remaining life-long inapparent carriers. Studies using immunosuppressive drugs, as well as studies in severe combined immunodeficient (SCID) foals have shown that adaptive immune responses, including CTL and neutralizing antibody, are required for EIAV control. Thus, EIAV infection in horses is a unique and powerful model system for investigating the correlates of protective adaptive immune responses against a naturally occurring lentivirus in an outbred species.

a.) Our research is focused on defining the correlates of CTL and neutralizing antibody-mediated protection against EIAV, so that an effective vaccine can be developed. We have defined CTL epitopes associated with protection, identified MHC class I molecules that present these epitopes, quantified CTL responses during acute and inapparent infection, and determined the qualitative characteristics of EIAV-specific CTL in terms of functional avidity. We also have identified factors contributing to viral envelope variation during EIAV infection, including CTL and neutralizing antibody selection pressure. Further studies are planned to dissect the minimum requirements for neutralizing antibody-mediated protection against EIAV by infusion of neutralizing equine monoclonal antibodies into SCID foals. The information gained should have important implications for developing effective vaccines against other lentiviruses, including HIV-1. In addition, our work should provide insight for developing protective vaccines against other important pathogens of horses.

b.) The equine industry has an annual impact on the U.S. economy of $102 billion. Therefore, equine health is economically important. Although the USDA has an EIA control program and the reported prevalence is low, the true prevalence is not known since less than 25% of the U.S. horse population is tested. Therefore, a significant reservoir population is possible. A recent EIA outbreak associated with high mortality in horses in Ireland, a country previously free of EIA, has shown that the disease can result in significant loss. Our research could lead to a better understanding of how to control EIAV infection, thus helping to protect the U.S. horse population. In addition, because our research is expanding the knowledge of equine immune responses, it could improve the ability to protect horses against a variety of other important pathogens, including equine herpes virus-1, equine influenza virus, equine arteritis virus, *Steptococcus equi*, and *Rhodococcus equi*. Defining the correlates of protection against a

lentivirus is relevant to biomedicine because it will have implications for developing a protective vaccine against HIV-1.

c.) Simian immunodeficiency virus (SIV) infection in rhesus macaques is thought to be one of the best models for HIV-1 infection in humans. However, EIAV infection in SCID foals (with selective immune reconstitution) allows dissection of protective adaptive immune responses against a naturally occurring lentivirus (in the natural host species) in a way that is unavailable in any other lentivirus model system.

d.) Although HIV-1 observational studies, clinical drug trials, and vaccine trials can be performed in humans and yield extremely important data, controlled studies using a defined virus challenge are not possible in humans. Therefore, EIAV studies in horses and SCID foals are superior for rigorously addressing hypotheses regarding the basic correlates of immune protection against lentivirus infection.

e.) Increased understanding of the equine immune response against persistent infections is needed. This knowledge will lead to more effective disease preventive strategies, including better vaccines. Protecting equine health is in the economic best interest of the United States, and should therefore be of interest to the USDA. The NIH is already funding EIAV research as a model for HIV-1. Therefore, joint funding between the NIH and USDA for equine immunology research, and specifically immune control of EIAV, is recommended.

Of Mice, Calf and Men: The Use of Domestic Species to Study Salmonella-induced Acute Intestinal Inflammation.

Andreas Baumler, Department of Medical Microbiology and Immunology, University of California, Davis, California

Area of research: A current limitation for studying innate immunity in the intestine is that the host response to infection observed in the most commonly used animal model, the mouse, differs dramatically from that observed in the human. For example, oral *Salmonella enterica* serotype Typhimurium infection in mice results in intestinal inflammation characterized by a slowly developing mononuclear infiltrate which develops in the absence of diarrhea. In contrast, *S.* Typhimurium infections in calves and humans are characterized by a rapidly developing neutrophilic infiltrate in the intestine that is always associated with diarrhea. A better understanding of how an acute inflammatory response in the intestine is orchestrated will require studies using relevant animal models.

Relevance to animal agriculture and biomedicine: Innate immune recognition of microbes in the intestine is a pivotal step during host pathogen interactions that result in disease. The rationale for studying how innate immune recognition in tissue triggers inflammation is that this research will provide insights into fundamental mechanisms by which the host mounts a protective response against microbial penetration of epithelial barriers at mucosal surfaces. A detailed knowledge about the mechanisms involved in the regulation of acute intestinal inflammation may identify new therapeutic targets for infectious diseases in animals and humans and will contribute to a fuller understanding of states of chronic inflammation, such as inflammatory bowel disease.

Advantages of domestic species as a translational biomedical model: Animal models are essential tools for studying the complex series of interactions between pathogens and different cell types *in vivo*, which ideally should result in the characteristic pathological changes observed in humans. Although mice are attractive model organisms for many investigations, because of low cost, availability of reagents and genetic manipulations, innate host responses observed in mice do not always reproduce accurately those in the human intestinal mucosa. As a result, some of the fundamental mechanisms leading to intestinal inflammation in humans have remained poorly understood. The bovine host closely resembles the human with regard to clinical manifestations, pathological changes and changes in host gene expression observed during infection. Characterization of the host response in calves is significant**,** because it will provide insights into responses that cannot be studied using mice.

Advantages over direct studies in humans: Animals provide the advantage of measuring host responses to infection in an experimentally well-controlled fashion. For example, the bovine ligated ileal loop model allows the collection of samples at defined time points of up to 12 hours after infection, thereby providing a powerful tool for studying host pathogen interaction *in vivo* during a critical window of infection.

Recommended focus area of research: We suggest research using relevant animal models to study host responses to invasion by human enteric pathogens *in vivo*. This approach will overcome limitations inherent to *in vitro* tissue culture models and *in vivo* rodent models. We expect that the outcome of this research will unveil mechanisms underlying variations in host response (previously established *in vitro* or using rodent models) that will help explain significant differences in disease outcome observed between mice and humans. The result will be an improved understanding of fundamental mechanisms controlling host responses to microbial invasion in the healthy human intestine.