BIOGRAPHICAL SKETCH

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NAME: Piedrahita, Jorge A

eRA COMMONS USER NAME (agency login): JPIEDRAHITA

POSITION TITLE: Director

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of British Columbia, Vancouver, British Columbia	BS	1981	Agriculture
University of California, Davis	MOTH	01/1984	Reproductive Physiology
University of California, Davis	PHD	01/1989	Cell and Developmental Biology
University of North Carolina Medical School	Postdoctoral Fellow	1991	Molecular Genetics

A. Personal Statement

My laboratory has been intimately involved in the isolation and characterization of a wide range of stem cells for the last 30 years and in the use of genomic tools for a range of biomedical applications. I originally trained in mouse ES cells under the direction of Dr. Gail Martin, one of the scientists who first described mouse ES cells. I continued this training with Dr. Oliver Smithies learning to genetically manipulate mouse ES cells by homologous recombination. Since starting my own lab in 1992 I have continued to work on genomics and stem cells in a wide variety of species and on the generation of genetically modified swine for use in regenerative medicine. My lab combines techniques in functional genomics, cell biology, embryo manipulation, and use of TALENs and CRISPR-Cas to generate complex gene edited pigs. In addition we have extensive experience with studying pigs in a wide variety of settings and experimental conditions.

B. Positions and Honors

Positions and Employment

1985 - 1988	Research on the isolation and characterization of mouse and porcine embryonic stem cells, in the laboratories of Dr. Gail Martin, Univesriy of California San Francisco and Dr. Gary B. Anderson, University of California-Davis, San Francisco and Davis, CA
1989 - 1991	Post-doctoral Research Fellow on homologous recombination in mouse embryonic stem cells, in the laboratories of Dr. Nobuyo Maeda and Dr. Oliver Smithies, University of North Carolina-Chapel Hill, Chapel Hill, NC
1991 - 1997	Assistant Professor, Department of Veterinary Anatomy and Public Health & Department of Animal Sciences, College Station, TX
1997 - 2002	Associate Professor, Department of Veterinary Anatomy and Public Health & Department of Animal Siciences, Texas A&M University, College Station, TX
1998 - 2002	Co-Director, Transgenic Core Facility, Center for Enviromental an Rural Health, Texas A&M University, College Station, TX
1999 - 2002	Associate Director , Center for Animal Biotechnology and Genomics, Texas A&M University, Colege Station, TX
2000 - 2002	Chair, Professional Program in Biotechnology, Texas A&M University, College Station, TX
2002 -	Professor, Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
2012 - 2015	Director, Center for Comparative Medicine and Translational Research, North Carolina State

	University, Raleigh, NC
2015 -	Director, Comparative Medicine Institute and Randall B. Terry Jr Distinguished Professor in Translational Medicine.

Other Experience and Professional Memberships

Honors

1992	Basil O'Connor Fellow, March of Dimes
1996	Faculty Research Award, Texas A&M University, Faculty of Genetics
1998	Award for Research Excellence, Pfizer
1999	International Senior Research Fellow, Fogarty
2000	Faculty Fellow, Texas A&M University
2002	Jack Green Endowed Professorship, Texas A&M University (Declined)
2006	Huffman Leadership Award, North Carolin State University
2006	Litwack Award, North Carolina State University
2009	Award for Research Excellence, Pfizer
2016	Distinguished Professorship. NC State

C. Contribution to Science

1. Role of Apolipoprotein E (ApoE) in atherogenesis. ApoE is a constituent of very low density lipoprotein and as such plays a critical role on cholesterol homeostasis. In humans variant forms of ApoE are implicated in familial type III hyperlipoproteinemia, a disease characterized by elevated levels of plasma cholesterol and premature coronary heart disease. In order to more carefully elucidate the role of ApoE in atherogenesis we developed a mouse deficient in ApoE by homologous recombination in ES cells. The mice, as expected, had elevated plasma cholesterol but more importantly developed foam-cell rich depositions in their proximal aortas. These lesions progressed until there was complete occlusion by 8 months of age. The ApoE knockout mouse demonstrated the importance of ApoE deficiency in the development of heart disease. Due to the slow progression of plaque formation this model has allowed multiple investigators to study many co-factors affecting the timing and severity of the disease. The importance of these models can be seen by the number of times the two original publications have been cited which at this time exceed 2,500 (741 and 1922, repectively; Google Scholar, Jan 20, 2015). My role in this work included the development of the mouse model by homologous recombination in mouse ES cells and participation in the initial analysis of the phenotype. This was one of the first mice made by homologous recombination in ES cells.

    a. Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci U S A. 1992 May 15;89(10):4471-5. PubMed PMID: 1584779; PubMed Central PMCID: PMC49104.
    b. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science. 1992 Oct 16;258(5081):468-71. PubMed PMID: 1411543.

2. Role of Folate binding protein 1 and 2 (Folbp1 and Folbp2) in neural tube defects. Folic acid supplementation reduces the occurrence of several human congenital malformations including craniofacial, and heart and neural tube defects. In order to better understand the mechanism by which folate influences neutral tube closure we generated mice that were deficient in either Folbp1 or Folbp2. Results indicated that while Folbp2 had no effect on neural tube formation, lack of Folbp1 resulted in incomplete closure of the neural tube and deal of fetus. Moreover, we demonstrated that the defect could be corrected by supplementation with high does of dietary folic acid. This was the first direct demonstration of the importance of a folic acid transporter on neural tube closure. These mice have been widely distributed and used by many investigators to continue to understand the role of folic acid in both normal and abnormal

physiology. In addition, these mutant mice have helped understand the role of folate in colon carcinogenesis, have helped clarified the lack of interaction of in utero arsenic exposure and folic acid transport, and the genetic bases of susceptibility to environmentally induced neural tube defects. My role in this project included participation in conceiving of the idea, generating the mice and their initial phenotypic analysis.

a. Piedrahita JA, Oetama B, Bennett GD, van Waes J, Kamen BA, Richardson J, Lacey SW, Anderson RG, Finnell RH. Mice lacking the folic acid-binding protein Folbp1 are defective in early embryonic development. Nat Genet. 1999 Oct;23(2):228-32. PubMed PMID: 10508523.
b. Finnell RH, Gelineau-van Waes J, Bennett GD, Barber RC, Wlodarczyk B, Shaw GM, Lammer EJ, Piedrahita JA, Eberwine JH. Genetic basis of susceptibility to environmentally induced neural tube defects. Ann N Y Acad Sci. 2000;919:261-77. PubMed PMID: 11083116.
c. Ma DW, Finnell RH, Davidson LA, Callaway ES, Spiegelstein O, Piedrahita JA, Salbaum JM, Kappen C, Weeks BR, James J, Bozinov D, Lupton JR, Chapkin RS. Folate transport gene inactivation in mice increases sensitivity to colon carcinogenesis. Cancer Res. 2005 Feb 1;65(3):887-97. PubMed PMID: 15705887; PubMed Central PMCID: PMC3938162.
d. Spiegelstein O, Gould A, Wlodarczyk B, Tsie M, Lu X, Le C, Troen A, Selhub J, Piedrahita JA, Salbaum JM, Kappen C, Melnyk S, James J, Finnell RH. Developmental consequences of in utero sodium arsenate exposure in mice with folate transport deficiencies. Toxicol Appl Pharmacol. 2005 Feb 15;203(1):18-26. PubMed PMID: 15694460; PubMed Central PMCID: PMC3938173.

3. Porcine Stem Cells. The mouse work described above clearly demonstrates the power of ES cells to generate complex transgenic animal models. As a result, my group focused its scientific efforts on isolating and characterizing a range of pig embryonic and primordial germ cell-derived (PGC) stem cells with the goal of obtaining pluripotent cell that could be used to generate complex genetic modifications in this species. The pig was chosen due to its physiological similarity to humans and its long history of utilization in biomedical research. Our work in this area led to the publication of the first descriptions of porcine ES cells (REF). Those initial, publications are still being cited today as they are considered classic papers in this field. While we were able to extensively study ES isolation in swine, none of the isolated cells lines could generate germ line chimeras. Today, 25 years later, that stills remains the case and no stable porcine ES cells are available. As a result my group turned to EG cells; ES-like cells derived from PGCs. This work led to the characterization of conditions for isolating and culturing porcine PGCs. In addition, my group was the first to demonstrate that porcine EG cells could generate chimeras and that the chimeras generated had contributions to the germ cells at the fetal stages. I continue to work with porcine stem cells, including induced pluripotent stem cells and have expanded the work to other species including the dog. My laboratory is internationally recognized as experts in a wide range of porcine stem cells.

a. Piedrahita JA, Anderson GB, Bondurant RH. On the isolation of embryonic stem cells: Comparative behavior of murine, porcine and ovine embryos. Theriogenology. 1990 Nov;34(5):879-901. PubMed PMID: 16726890.
b. Piedrahita JA, Moore K, Oetama B, Lee CK, Scales N, Ramsoondar J, Bazer FW, Ott T. Generation of transgenic porcine chimeras using primordial germ cell-derived colonies. Biol Reprod. 1998 May;58(5):1321-9. PubMed PMID: 9603271.
c. Lee CK, Weaks RL, Johnson GA, Bazer FW, Piedrahita JA. Effects of protease inhibitors and antioxidants on In vitro survival of porcine primordial germ cells. Biol Reprod. 2000 Sep;63(3):887-97. PubMed PMID: 10952936.
d. Koh S, Thomas R, Tsai S, Bischoff S, Lim JH, Breen M, Olby NJ, Piedrahita JA. Growth requirements and chromosomal instability of induced pluripotent stem cells generated from adult canine fibroblasts. Stem Cells Dev. 2013 Mar 15;22(6):951-63. PubMed PMID: 23016947; PubMed Central PMCID: PMC3585736.

4. Comparative Analysis of Genomic Imprinting. Our studies on ES cells and with mutations affecting early fetal development (Folbp1 and Folbp2) led my group to the study of genes that play a key role in fetal development and placental function. Imprinted genes have the unique characteristic of being expressed mono-allelically (paternal or maternal allele, but not both) and have been shown to play a key role in

energy distribution between the mother and the developing fetus. While these genes had been studied extensible in mice, and to a lesser extent in humans, there was little information available as to their conservation in other mammalian species. As a result, my group turned to studying this family of genes in two species, the bovine and the porcine. This work led to the demonstration that while here was a high degree of conservation between species there where some genes that were imprinting differed between species. Our work in pigs, in particular, remains to this day the most complete characterization of imprinted genes in this species and is a highly cited paper in the field. In addition, our studies in domestic animals led us to the study of placental-associated diseases in humans. This was done to try to determine whether imprinted genes were involved in two diseases; preeclampsia and uterine growth restriction/small for gestation age. Global gene expression analysis of large number of human at term placentas helped us elucidate the role of sialic acid and autoimmunity in preeclampsia. In addition, we were able to demonstrate that IUGR was a highly heterogeneous disease.

a. Dindot SV, Farin PW, Farin CE, Romano J, Walker S, Long C, Piedrahita JA. Epigenetic and genomic imprinting analysis in nuclear transfer derived Bos gaurus/Bos taurus hybrid fetuses. Biol Reprod. 2004 Aug;71(2):470-8. PubMed PMID: 15044262.
b. Bischoff SR, Tsai S, Hardison N, Motsinger-Reif AA, Freking BA, Nonneman D, Rohrer G, Piedrahita JA. Characterization of conserved and nonconserved imprinted genes in swine. Biol Reprod. 2009 Nov;81(5):906-20. PubMed PMID: 19571260; PubMed Central PMCID: PMC2770020.
c. Tsai S, Hardison NE, James AH, Motsinger-Reif AA, Bischoff SR, Thames BH, Piedrahita JA. Transcriptional profiling of human placentas from pregnancies complicated by preeclampsia reveals disregulation of sialic acid acetylesterase and immune signalling pathways. Placenta. 2011 Feb;32(2):175-82. PubMed PMID: 21183218; PubMed Central PMCID: PMC3039036.
d. Guo L, Tsai SQ, Hardison NE, James AH, Motsinger-Reif AA, Thames B, Stone EA, Deng C, Piedrahita JA. Differentially expressed microRNAs and affected biological pathways revealed by modulated modularity clustering (MMC) analysis of human preeclamptic and IUGR placentas. Placenta. 2013 Jul;34(7):599-605. PubMed PMID: 23639576; PubMed Central PMCID: PMC3677766.

5. Somatic Cell Nuclear Transfer (SCNT) and Transgenesis in Swine. Generation of complex genetically modified pigs continued to be a huge challenge due to the inability to generate stable ES or EG cells that could be used for genetic manipulation, and the limitations and costs of random insertion by pronuclear injection. As a result, with the announcement of the birth of Dolly, the first mammal generated by somatic cell nuclear transfer, we turned our efforts to successfully clone swine by SCNT. While our group was not the first one to do so, we contributed significantly to both improving the technology and to studying the effects of SCNT in pigs. Our work was the first to demonstrate that “cloned” pigs had significant epigenetic variability and that unlike other species such as mice, cattle, and sheep, fetal size was minimally affected. In addition, we combined genetic modification of donors cell and SCNT to generate transgenic swine. In the last three years we have now combined SCNT, with both Tal effecter nucleases and Crispr-Cas and have generated a range of transgenic swine including; a) swine carrying an H2B-GFP in the actin locus; these pigs will be used for tracking injected stem cells; b) a porcine model of dwarfism by inactivation of the HMGA2 gene. The generated pigs are on average 30% the size of wild type litter mates. c) Under HL51587 we have generated severe combined immuno deficient pigs by inactivation of the IL2RG and RAG1 genes. The generated animals lack B-cell, T-cell, and Nk-cells. In addition we have been able to engraft them with both allogenic pig cells as well as with xenogenic human CD34 hematopoietic stem cells. Those studies are ongoing. In short, my groups has been able to develop and apply the areas of SCNT and meganuclease-based technologies to generate a range of complex genetically modified pigs that can be used in biomedical research. These models will be widely distributed so other investigators can use them to make key discoveries in multiple fields. Our group remains focused on stem cell and their use in regenerative medicine.

a. Walker SC, Shin T, Zaunbrecher GM, Romano JE, Johnson GA, Bazer FW, Piedrahita JA. A highly efficient method for porcine cloning by nuclear transfer using in vitro-matured oocytes. Cloning Stem Cells. 2002;4(2):105-12. PubMed PMID: 12171703.
b. Estrada J, Sommer J, Collins B, Mir B, Martin A, York A, Petters RM, Piedrahita JA. Swine generated by somatic cell nuclear transfer have increased incidence of intrauterine growth restriction (IUGR). Cloning Stem Cells. 2007 Summer;9(2):229-36. PubMed PMID: 17579555.

c. Sommer JR, Jackson LR, Simpson SG, Collins EB, Piedrahita JA, Petters RM. Transgenic Stra8-EYFP pigs: a model for developing male germ cell technologies. Transgenic Res. 2012 Apr;21(2):383-92. PubMed PMID:

d. Sper RB, Koh S, Zhang X, Simpson S, Collins B, Sommer J, Petters RM, Caballero I, Platt JL, **Piedrahita JA**. Generation of a Stable Transgenic Swine Model Expressing a Porcine Histone 2B-eGFP Fusion Protein for Cell Tracking and Chromosome Dynamics Studies..Plos One 2017. 12:e0169242.PMID 28081156

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41158550/?sort=date&direction=descending

D. Ongoing Research Support

ACTIVE

1 R03 AR068112-01 (PI: Fisher) 04/01/2015 - 03/31/2018 0.12 calendar months
NIH $150,000
Age-Dependent ACL Function during Growth: Guiding Injury Treatment in Children
The major goal of this project is to utilize a surrogate animal model to study the age-dependent function of the ACL and its bundles in the growing joint as well as the impact of partial or complete ACL injury.

T32 OD011130 (PI: Jones) 08/01/2008 - 07/31/2018 0.01 calendar months
NIH/OD $394,623
Comparative Medicine and Translational Research
Training grant

R21 R21 OD019738 (PI: Piedrahita) 07/**15**/2014 - 06/30/**2017** 0.94 calendar months
NIH/OD $150,000
Improved Large Animal Model for the Study of Adult Stem Cells
Development of a transgenic pig for the study of intestinal stem cells.

G20OD020279 (PI: Piedrahita) 04/01/2015 - 03/31/**2017** 0.0 calendar months
NIH $493,534
Housing and Analysis Facilities for Miniature and Transgenic Swine: A Biomedical Resource
The overall aim of this equipment grant is to develop new capabilities for the housing and analysis of miniature and/or transgenic swine requiring BSL2 containment

06-S150670 (PI: Piedrahita) 03/15/2015-02/29/2017 0.0 calendar months
Texas A&M / Foundation for Angelman Syndrome Therapeutics $75,000
Development and Characterization of a Pig Model of Angelman Syndrome

Unassigned (PI: Piedrahita) 04/01/2015-11/30/2017 1.2 calendar months
Vesta Therapetucs / UNC Chapel Hill $201,005
Grafting Strategies for Liver Cell Therapies
Proposal to Characterize the Hepatic and Pancreatic Duct stem cells in pigs at different stages of development.