Cloning &Transgenic Swine for Medical and Agricultural Uses at MU

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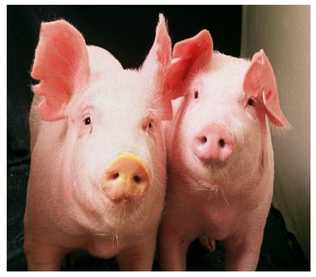
NSRRC National Swine Resource & Research Center

College of Agriculture Food and Natural Resources

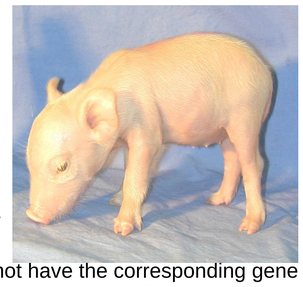
The pig is an important component of the world's food supply. With an inventory of 66.7 million head (1), the United States is the world's 3rd largest producer and 2nd largest consumer, exporter, and importer of pork and pork products. Total farm income for hogs in 2008 has estimated to be $16.0 billion (3). Not only are pigs important to agriculture they are important in biomedical research as they are excellent models for cardiovascular disease, atherosclerosis, cutaneous pharmacology, wound repair, cancer, diabetes, ophthalmology, toxicology research, lipoprotein metabolism, pathobiology of intestinal transport, injury and repair, as well as being considered potential sources of organs for xeno-transplantation. Furthermore, the swine genome is also similar to the human, as a phylogenetic approach using swine genome sequence data shows that the swine genome is 3x closer to the human than is the mouse. Reviewers at the NIH consider swine to be a very important model for human health and disease as evidenced by the fact that for the past 6 years extramural support of research on swine has averaged over $115 million per year (NIH Office of the Director). The NIH considers swine to be so important that it has helped establish the National Swine Resource and Research Center at the University of Missouri (4) to serve as a genetic resource for the biomedical community.

February 29, 2000
1st Transgenic pigs from an in vitro system: the enhanced Green Fluorescent Protein (eGFP) via oocyte transduction (Cabot et al., 2001).
"Patty and Duffy"



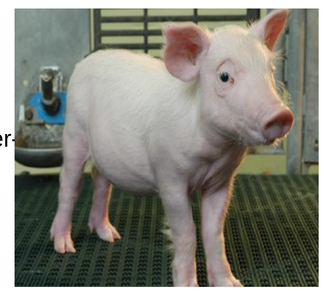
March 9, 2001 1st Cloned Transgenic pigs created by somatic cell nuclear transfer (they also contain the eGFP construct: Park et al., 2001).

September 21, 2001
1st Gene Knockout. α-1, 3-galactosyltransferase. Important for Xeno-transplantation (Lai et al., 2001). The presence of galactose a(1,3) galactose residues (GGTA1) on the surface of pig cells is a major obstacle to successful xeno-transplantation. Humans do not have the corresponding gene and therefore produce preformed natural antibodies against this epitope. This is responsible for hyperacute rejection of porcine organs.



November 18, 2002
1st homozygous Knockout miniature pig (α-1,3-Galactosyltransferase: Kolber-Simonds et al., 2004).

"Goldie"



July 29, 2002+
1st Transgenic pigs with a liver-specific promoter driving a suicide gene (Beschorner et al., 2003a,b). It should be possible to take hepatocytes from a human, genetically modify them to fix any defect and then introduce them into a preimmune fetal pig. The human cells should colonize the liver of the pigs. When the pigs reach adult size the pig liver cells can be selectively destroyed, leaving a human liver.
"Nip & Tuck"

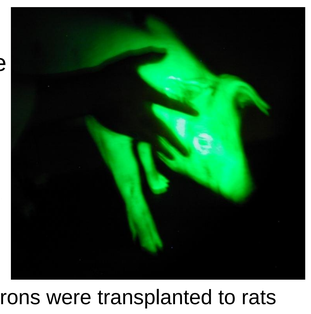
February 12, 2003+
Spot clones born as a result of testing different methods for cloning showed that some clones are immuno-compromised (Carroll et al., 2005).

July 6, 2004
1st Gene Knockout (α-1,3-galactosyltransferase) and transgene for human decay accelerating factor (hDAF). hDAF will help prevent compliment-mediated cell lysis even if antibody will bind to the cells (Lai et al., unpublished).
"Dafney"

June 24, 2005 Pigs transgenic for the hFat-1 gene. *Fat-1* is a gene from *C. elegans* that coverts *n*-6 fatty acids to *n*-3 fatty acids. The pigs produce higher levels of n-3 fatty acids as compared to n-6 fatty acids.. These animals will allow researchers to determine the effects of Omega 3 fatty acids on the health and wellbeing of the pigs. In addition, if similar animals are permitted to enter the food chain eating this potentially heart healthy pork may have a positive effect on human health (Lai et al., 2006).

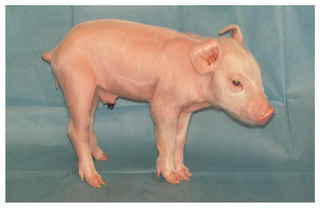
November 17, 2005 1st Cryopreserved transgenic cloned piglets born. The ability to cryopreserve the embryos of pigs will greatly facilitate the movement of these valuable genetics between different institutions (Li et al., 2006).

April 12, 2006
More eGFP pigs born that have even better expression levels (Whitworth et al., 2009). Cells from these 'green' pigs were used for transplantation studies to Evaluate how transplanted cells re-populate a damaged retina (Klassen et al., 2008), and to confirm that stem cells were contributing to the brains when neurons were transplanted to rats (Price et al., 2006).



Also see National Geographic July 2005, page 18.

May 30, 2006
1st CFTR KO born. Designed to create a model of cystic fibrosis, which is the most common genetic disease in North American adolescents (Rogers et al., 2008a).



July 16, 2006+
Provided clones of TJ Tabasco to the University of Illinois so that they have the animals that have been sequenced for their research program (Hao et al., unpublished).

June 16, 2007
1st Pigs born as a result Of In Vitro produced blastocysts that were cryopreserved (Li et al., 2009).

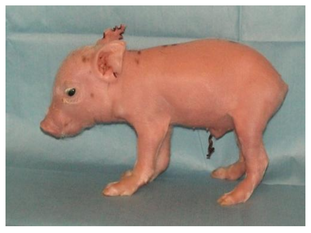
July 19, 2007
1st ΔF508 CFTR pigs born. The ΔF508 mutation exists in 70% of the people With Cystic Fibrosis (Rogers et al., 2008a).

August 13, 2007
1st Cloned pigs derived from skin-stem cells (Hao et al., 2009).

February 27, 2008
1st homozygous CFTR KOs born. These pigs get all the symptoms of cystic fibrosis (Rogers et al., 2008b).

March 10, 2008
Human Coagulation Factor VIII, SERPINA1 & Von Willebrand Factor pigs born Zhao et al., unpublished). To isolate pharmaceuticals from to treat people with hemophilia.

May 26, 2008
P23H Rhodopsin Miniature pigs born (Ross et al., Unpublished). The P23H Mutation is the most common form of Retinitis Pigmentosa.



June 2, 2008
eGFP Yucatan Miniature pigs born (Whyte et al., unpublished).

September 22, 2008
Human Coagulation Factor FIX, SERPINA1, & PACE pigs born Zhao et al., unpublished). To isolate pharmaceuticals from to treat people with hemophilia.

March 16, 2009
Tie2-Catalase/eGFP pigs born. Designed to reduce levels of nitric oxide to study cardiovascular disease. (Whyte et al., unpublished).

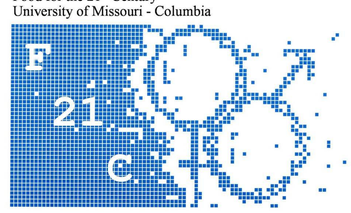
NSRRC

Import and rederiveration of Valuable Models, Infectious Disease Monitoring, Cryopreservation, Genetic Modification to Create New Models, Assisted Reproductive Technology

Provide Service, Training and Research. Serve as a Genetic Resource and as a Transgenic Swine Facility Core for the NIH.

Animal Reproductive Biology Group

Food for the 21st Century
University of Missouri - Columbia



November 2, 2009
Tie2-eNOS/ eGFP Yucatan Miniature pigs born (Whyte et al., unpublished). These pigs will be used to study cardiovascular disease.

November 16, 2009
IFABP-CFTR on a CFTR-/ background. These pigs Meet out goal of a Cystic Fibrosis model that does Not have an obstructed bowel (Welsh et al. unpublished).

Current work is to create a model of Spinal Muscle Atrophy and Juvenile onset of Diabetes, to create a pig that is not susceptible to PRRSV, eNOS knockouts, and Interferon αβ Receptor KOs.

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