Regenerative Medicine Minnesota
Final Report
Due: 2/28/2018

Grant Title: Generating human neural stem and progenitor cells in a porcine model through blastocyst complementation

Grant Number: RMM 11215 TR003

Principal Investigator: Ann Parr, PhD, MD

Project Timeline: 2/1/2016 - 1/31/2018

Progress to Date:
Aim 1. To create a porcine knockout for SOX2 and demonstrate that this model can be utilized to create human neural stem cells (hNSCs), utilizing blastocyst complementation with either: 1a. CD34 negative non-hematopoietic human umblical cord blood stem cells (hUCBSCs), or 1b. human induced pluripotent stem cells (hiPSCs).

Aim 2. To create a porcine knockout for OLIG1/OLIG2 and demonstrate that this model can be utilized to create human oligodendrocyte progenitor cells (hOPCs), utilizing blastocyst complementation with either: 2a. CD34 negative non-hematopoietic human umbilical cord blood stem cells (hUCBSCs), or 2b. human induced pluripotent stem cells (hiPSCs).

The goal of this project was to develop a large animal model to generate a source of human oligodendrocyte precursor cells that could potentially be isolated and transplanted into demyelinated patients. The NIH has enacted a moratorium on human-animal chimeras, so we pursued pig-to-pig chimeras. Finally, we focused on the OLIG1/OLIG2 chimera model (AIM 2), as we found this model generated a neural stem cell population upon complementation.

Please list any of the following that have resulted from the Minnesota Regenerative Medicine grant funding:

Voth JP, Miller ZD, Pengo T, Steevens AR, Danczyk GR, Webster DA, Calrson DF, Low WC, Parr AM. Exogenic intraspecies porcine embryonic stem cells integrate into an OLIG1/OLIG2 knockout porcine blastocyst and exhibit a neural stem cell phenotype. Submitted to Scientific Reports on June 21, 2018. Under review.

Budget Update:
The budget was utilized as described in the proposal.

Reporting to all Minnesotans:

Oligodendrocytes are the myelinating cells of the brain and spinal cord, functioning similarly to the rubber coating on a wire. When these cells are lost, our neurons cannot communicate properly, leading to disabilities and illnesses such as multiple sclerosis and spinal cord injury, among many others. Current treatments for these illnesses largely treat symptoms without reversing or resolving the damage that is already done. In the last ten years, stem cell therapies have been studied to restore the lost oligodendrocytes, but to date these methods have only been marginally successful. Our approach to this problem is to use the developing pig as a "biological incubator" to develop oligodendrocytes that are likely more similar to the patient's oligodendrocytes than other methods to create oligodendrocytes in a dish. This funding allowed us to successfully generate one pig to pig chimeric organism, where we introduced pig cells into the very early developing pig embryo with a genetic mutation to eliminate the embryo's own oligodendrocytes. We attempted to remove the pig's own oligodendrocytes so that our introduced pig stem cells would preferentially fill the "gap" we created with the knockout. We found our introduced pig stem cells survived in the late first trimester pig embryo, and even developed into neural stem cells. This is a promising result as neural stem cells ultimately give rise to oligodendrocytes later in development. We are very excited with these results, as they introduce options of producing oligodendrocytes that could be used for transplantation into multiple sclerosis or spinal cord injury patients. A key aspect of this approach is that these human oligodendrocytes would be derived from the patient's own cells, so there would be no risk of immune rejection. Our findings are currently submitted to *Scientific Reports* and in the process of being peer-reviewed.