

**BIOMEDICAL & VETERINARY SCIENCES
GRADUATE PROGRAM**



ANNOUNCES

The Doctor of Philosophy Seminar
and Examination of

Maria Cristina Villafranca L.

**“Fusion of bovine fibroblasts to mouse
embryonic stem cells: a model to study
nuclear reprogramming”**

**Thursday, March 22, 2018
10:00 am
VMIA Classroom 220**



Maria Cristina and Carey, a trained VT PAWS therapy cat.

Funded by

VMCVM Office of Research and Graduate Studies

Virginia Tech Institute for Critical Technology and Science (ICTAS)

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(IGEP) in Regenerative Medicine

Lay Language Abstract

The cells of an early embryo have the potential to give rise to any cell type found in the adult body. When these cells are transferred to a culture dish and kept under the right conditions, they become Embryonic Stem Cells (ESCs), and they retain the same developmental potential as the original embryonic cells they were derived from. In 2006, researchers in Japan found that it is possible to “reprogram” the cells of an adult individual (for example, fibroblast skin cells taken from a biopsy) to an embryonic state, by forcing the cells to express extra copies of genes that are normally active in embryos. These reprogrammed cells are called induced Pluripotent Stem Cells (iPSCs), and similarly to ESCs, they also have the potential to produce any cell type found in an adult organism. Lines of iPSCs from livestock species have potential applications in agriculture, species conservancy, biomedical industry, and veterinary and human health. Unfortunately, for reasons that are to date not fully understood, the technology to produce iPSCs has, so far, only worked in mice and humans.

We first attempted to produce bovine iPSCs by adapting methods and conditions used to derive iPSCs in mice and humans. We observed partial reprogramming of bovine cells, but were ultimately not able to produce true bovine iPSCs. This suggests that the bovine requires alternative/additional factors to induce reprogramming in adult cells. However, not knowing exactly what conditions or reagents will induce the reprogramming process in the cow, we decided to take a different approach. We focused on trying to understand how nuclear reprogramming works in the bovine. This would allow us to rationally target the specific requirements of potential bovine pluripotent cells.

It is known that the fusion (“merging”) of an adult cell with a stem cell, causes the adult cell to change its gene expression pattern to resemble a stem cell. We therefore fused mouse ESCs with bovine fibroblasts, and observed changes in bovine gene expression pattern as early as 24 hours after fusion. The gene expression changes observed resemble those found during early reprogramming of human and mouse iPSCs, and are accompanied by silencing of fibroblast specific genes. This suggests that our cell fusion model recreates the changes that happen during reprogramming, and can therefore be used as a tool to better understand pluripotency in the cow. The cell fusion method that we describe in this work can in theory also be adapted to other species, by

fusing somatic cells from other species to mouse ESCs, and finding species specific relevant pluripotency genes.

Publications

Garrido Bauerle, M.J., Morera Galleguillos, F., and **Villafranca Locher, M.C.** (2017). Identification of interspecific mouse-bovine heterokaryons using indirect immunofluorescence. *Universidad Austral de Chile*.

Saldivia Barria, M.I., **Villafranca Locher, M.C.**, and Morera Galleguillos, F. (2014). Design and cloning of a lentiviral vector containing a monomeric red fluorescent protein (mRFP) reporter gene to label mouse embryonic stem cells. *Universidad Austral de Chile*.

Inostroza Caceres, L.X., **Villafranca Locher, M.C.**, and Gomez Jaramillo, M. (2014). Analysis of EphA4 gene expression in murine brain endothelial cells. *Universidad Austral de Chile*.

Villafranca Locher, M.C. (2013). Editor's choice: Cell culture. *Silhouette Lit. Art Mag.* 34–35.

Villafranca Locher, M.C., Ratto Fuster, M.H., and Peralta Troncoso, O.A. (2009). Quantification of the cellular prion protein (PrPC) mRNA in bovine gametes and during bovine embryo development. *Universidad Austral de Chile*.

Presentations

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells. Poster at 29th Annual BMVS Graduate Research Symposium. Blacksburg, VA. 03/2018

Villafranca MC. Understanding pluripotent stem cells by fusing mice and cows: Can this model help us improve human regenerative medicine? Talk at 2017 Advancing the human condition symposium. Blacksburg, VA. 11/2017

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells. Talk at 33rd Annual Virginia Tech Graduate Student Assembly Research Symposium. Blacksburg, VA. 03/2017

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine

fibroblasts to pluripotency by fusion to mouse embryonic stem cells.
Poster at 28th Annual Research Symposium. Blacksburg VA. 03/2017

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells.
Poster at 2016 World Stem Cell Summit. West Palm Beach FL. 12/ 2016

Villafranca MC, Williams KR, Eyestone WH. Reprogramming Bovine Fibroblasts to Pluripotency by Fusion to Mouse Embryonic Stem Cells.
Poster at Large Animal Genetic Engineering Summit. Bethesda, MD.
09/2016. - received travel award

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells.
Poster at 27th Annual Research Symposium. Blacksburg VA. 03/2016

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells
Poster at GSA Research Symposium. Blacksburg VA 02/2016

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells.
Poster at 2015 World Stem Cell Summit. Atlanta, GA. 12/2015

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells.
Poster at Regenerative Medicine Retreat. Mountain Lake Lodge, VA.
05/2015

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells
Poster at GSA Research Symposium. Blacksburg VA 03/2015

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells.
Talk at 26th Annual BMVS Graduate Research Symposium. Blacksburg VA. 03/2015

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells.
Poster at Regenerative Medicine Retreat. Mountain Lake Lodge, VA.
05/2014

Villafranca MC, Williams KR, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells.

Poster at 25th Annual BMVS Graduate Research Symposium.
Blacksburg, VA. 03/2014

Villafranca MC, Eyestone WH. Reprogramming bovine fibroblasts to pluripotency by fusion to mouse embryonic stem cells. Poster at 24th Annual BMVS Graduate Research Symposium. Blacksburg, VA. 03/2013

Villafranca MC, Peralta OA, Eyestone WH. Cuantificación del transcrito de la proteína celular del prion (PrPC) en gametos y durante el desarrollo embrionario bovino. Poster at Congreso Veterinario de Leon. Leon, GTO. 09/2012 - first place poster presentation

Villafranca MC, Eyestone WH. Identification of pluripotency factors using a heterospecific cell fusion approach. Poster at 23rd Annual Research Symposium. Blacksburg, VA. 09/2011

Villafranca MC. Cuantificación del transcrito de la proteína celular del prion (PrPC) en gametos y durante el desarrollo embrionario bovino. DVM final examination seminar at Universidad Austral de Chile. Valdivia, Region de los Rios. 10/2009

Villafranca MC, Peralta OA, Eyestone WH. Quantification of endogenous prion gene expression in bovine gametes and preimplantation embryos. Poster at Merck-Merial-NIH National Conference . Bethesda, MD. 08/2007

Awards and Academic Achievements

First place winner team. One Health Day competition. Virginia-Maryland College of Veterinary Medicine. Blacksburg, VA. 11/2017

Travel award to present at Large Animal Genetic Engineering Summit. Bethesda, MD. 09/2016

Interdisciplinary Graduate Education Program (IGEP) travel fund 2016

Graduate Student Assembly (GSA) travel fund 2015

Research and Graduate Studies (RGS) travel fund 2012, 2015, 2016

First place poster presentation at Congreso Veterinario de Leon. Leon, GTO. 09/2012 2016-2017

Examination Graduate Committee

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Will Eystone, PhD
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