A251 Cloning, Transgenesis and Stem Cells

Porcine putative primordial germ cells-likes (ipPGCL) generated from induced pluripotent stem cells (piPSCs)

N.C.G. Pieri[1], L.S. Machado[2], A.F. Souza[3], R.C. Botigelli[4], F.V. Meirelles[2,3], F.F. Bressan[2,3], A.F.C. Andrade[1]

[1]FMVZ/ USP - Swine Research Center, Faculty of Veterinary Medicine and Animal Sciences, Pirassununga, SP, Brasil; [2]FMVZ/ USP - Department of Surgery, Faculty of Veterinary Medicine and Animal Sciences, São Paulo, SP, Brasil), [3]FZEA/USP - Department of Veterinary Medicine, Faculty of Animal Sciences and Food Engineering, Pirassununga, SP, Brasil; [4]UNESP - São Paulo State University, Institute of Biosciences, Department of Pharmacology, Botucatu, SP, Brasil.

Recent studies in murine model have reported that induced pluripotent stem cells (iPSCs) are able to different into primordial germ cell-like (PGCL) (Hayashi et al. 2011 Cell 146, 519-532); however, no data is already reported in domestic animal models. The porcine is an important model for translational and regenerative research due to its similar physiological, anatomical and morphological features to humans. For this reason, the generation of porcine PGCLs *in vitro* (ipPGCL) can be useful to understand genetic and epigenetic remodeling of these cells *in vitro* and *in vivo*. This study aimed the generation and characterization of porcine primordial germ cells-like (ipPGCL) *in vitro*. First, porcine induced pluripotent stem cells (piPSCs) were induced into epiblast-like cells (EpiLC) by culture in fibronectin-coated (16.7mg/ml) 6-well plates and N2B27 culture medium supplemented with 20ng/ml activin A, 12ng/ml basic fibroblast growth factor (bFGF), and 1% knockout serum replacement (KSR) for 48 h. Then, epiblast-like cells were induced to differentiation in ipPGCL by non-adherent culture (Agree well plates, StemCell Technologies, Vancouver, BC, Canada) with GK15 medium (GMEM) supplemented with 15% KSR, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 0.1 mM 2-mercaptoethanol, 2mM L-glutamine, 1% antibiotics, 500ng/ml BMP4, 100 ng/ml SCF, 500ng/ml BMP8b, and 50ng/ml epidermal growth factor for 4 days. Lastly, putative ipPGCLs were characterized according to its morphology, alkaline phosphatase detection and expression of OCT4 and gem cells (DDX4/VASA, DAZL) markers. Our results showed that piPSCs can be induced into EpiLCs and these cells were further induced into putative ipPGCLs. In addition, ipPGCLs had typical morphological features: oval or round shape and irregular contour, were positive for alkaline phosphatase, OCT4, DDX4 (VASA) and DAZL. These preliminary data represent the first step for *in vitro* generation of porcine ipPGCLs. The ability to generate ipPGCLs from piPSCs may provide an adequate *in vitro* model to be used in the study of unanswered questions about germ cell biology and infertility.
Financial support: FAPESP 2015/25564-0 and 2015/26818-5.