

Plan Overview

A Data Management Plan created using DMPTool-Stage

Title: How could we manage to differentiate cattle iPSC into PGCs and functional gametes?

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Project abstract:

The effective generation and use of induced pluripotent stem cells (or iPSCs) in different species have the great advantage of generating cells with pluripotent characteristics, which can be genetically edited or not, and are able, through germline induction, of developing and differentiate into gametes with known genetic background, and also creating an ideal platform for in vitro and in vivo modeling of several syndromes. Primordial germ cell-like cells (PGCLCs) generated from iPSCs in vitro have been proven successful in the mice model as gametes precursors and seem likely to provide broad applications using germinative cells in other species in the future. The precise detection of cell fate decision mechanisms is crucial in the reprogramming process, revealing effective and involved processes such as cell signaling pathways, spatial and temporal gene expression, and epigenetic modification. PGC formation and gametogenesis is a transient period in mammal's life characterized by specific events influenced by genetic and epigenetic mechanisms, including transcripts encoding proteins that implement particular types of biological functions reflecting coordinated control at the transcriptional and posttranscriptional levels. Germ-plasm components and cell signaling molecules encoded in mRNAs will be identified and analyzed by RNAseq technique; moreover, the methylation analysis, as upstream operator of gene expression, will be crucial to understand gene expression patterns of DNA or histone modifications in iPSC and derived PGCLCs. This proposal aims to establish authentic indicators and criteria to validate the characterization of iPSC-derived PGCLCs and then induced gametes through molecular genetics, bioinformatics, and epigenetic techniques (RNAseq, global and specific DNA methylation analysis).

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How could we manage to differentiate cattle iPSC into PGCs and functional gametes? - Coleta de Dados

Serão analisados dados morfológicos e moleculares a partir da coleta, isolamento e caracterização de células reprogramadas à pluripotência e diferenciadas *in vitro* em EpiCLs e PGCs, bem como os dados da especialização das PGCs em gametas femininos. Todos os dados a serem analisados serão desenvolvidos durante o projeto.

Os dados relacionados à morfologia celular serão analisados por fotodocumentação das células com EVOS™, os dados de detecção de proteínas derivados de imunocitoquímica serão coletados a partir de fotodocumentação em microscópio confocal e análise no software *ImageJ*, e os dados moleculares (expressão gênica) serão gerados a partir de RT-qPCR ou perfil de miRNAs no *7500 Real-Time PCR System™*, ou ainda, através de análise de transcriptoma.

Todas as análises estatísticas serão realizadas pelo programa estatístico *Statistical Analysis System (SAS University Edition)* e todos os dados, em especial relacionados à transcrição de genes, poderão ser compartilhados e reanalisados em outros projetos. Todos os experimentos serão feitos com repetição para garantia da análise.

Dessa forma, os dados gerados a partir deste estudo vão permitir a comparação de protocolos inter-espécies e de maneira inédita contribuir para o estudo translacional do desenvolvimento das linhagens germinativas *in vitro*.
