A206 Cloning, transgenesis and stem cells

Transfection of swine oocyte with polyethyleneimine (PEI): a low cost and convenient method to produce genetically modified swine

Andressa Pereira de Souza[2,3], José Rodrigo Pandolfi[1], Emanuelle Coldebella[3], Shaiana Salete Maciag[3], Francisco Noé da Fonseca[1], Carlos André da Veiga Lima Rosa[2], Mariana Groke Marques[1]

[1]Embrapa Suínos e Aves - Embrapa Suínos e Aves, Concórdia, SC, Brasil; [2]UDESC/CAV - Universidade do Estado de Santa Catarina, Lages, SC, Brasil; [3]IFC - Concordia - Instituto Federal Catarinense - Campos Concordia, Concórdia, SC, Brasil.

Oocytes are excellent candidates to produce genetically modified pigs due to their physiology and absence of nuclear envelope, which favors the incorporation of DNA. However, the presence of the zona pellucida and the sensitivity to stressors make transfection a challenge, since the techniques available are labor-intensive and expensive. Therefore, the objective of this work was to develop a protocol for transfection of porcine oocytes using a cationic polymer, polyethyleneimine (PEI). Thus, the branched PEI 25 KDa (100mL, Sigma Aldrich, Saint Louis, USA) was used. Oocyte maturation and in vitro embryo production procedures were performed according to Marques *et al.*, 2011 (Zygote, 19: 331-337). The data (mean minimum squares ± SE) were evaluated using PROC MIXED (SAS®) with 5% significance. In the 1st experiment, the ability of PEI to overcome the zona pellucida and the cytoplasmic membrane of oocytes matured in vitro was evaluated. For that, PEI was labeled with FITC, and oocytes were incubated (30 min) with 4 concentrations of PEI-FITC (10, 20; 40 and 80 μg/mL). The internalization of the PEI-FITC was evaluated by fluorescence microscopy and the pixel quantification performed using the software Image J 1.40g®. It was observed that all concentrations of PEI were able to reach the cytoplasm. The internalization rate was significant ($p<0.001$) and concentration dependent, and the concentration 10 μg/mL resulted in the lowest internalization as the concentration of 80 μg/mL provided the highest one ($19.60\pm0.25x10^3$ and $22.69\pm0.23x10^3$ pixels, respectively). In the 2nd experiment, transfection rates were evaluated using two preparations containing PEI (20 or 80 μg/mL) complexed with the pmhyGENIE-5 vector at 2 N/P ratio, and then incubated with oocytes matured in vitro. Incubations with the respective vector concentration were also performed in the absence of PEI (INC20 and INC80) and a Control group. After 30 min of incubation, the oocytes were fertilized and cultured in vitro until day 7 of development. No effect of the treatments on the cleavage rates ($p=0.8307$) and blastocysts ($p=0.9780$) were observed. The cleavage rates ranges from 41.19±10.55% to 54.83±7.46% and the blastocyst rates from 16.96±7.81% to 23.68±7.81%. Besides, only the PEI20 group presented blastocytes with GFP expression (3.2±1.91%). The data suggest that PEI, unlike other transfectant agents, has the ability to pass the zona pellucida, and the protocol described herein is capable of producing transgenic blastocysts expressing GFP, so that it could be used as a cheap and easy tool for transfection of swine oocytes.