A243 Cloning, Transgenesis and Stem Cells

## Polyethyleneimine (PEI) as polyfection system for swine sperm

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For the generation of transgenic animals, the insertion of exogenous DNA into the cell or gamete is crucial. There are several methods for cell transfection, most of which are expensive, poorly effective or both. Cationic polymers, including PEI, bind to DNA, forming PEI-DNA complexes. These, bind to the cell surface, interact with the cell membrane and are internalized by endocytosis. Once inside the cells, the PEI-DNA complexes are protected by the endosome, and then migrate to the nucleus, where the DNA molecules are released. However, PEI polyplex itself has not been tested on sperm of any animal species. Thus, this study aimed to develop a protocol for transfecting swine sperm using PEI as polyfection agent. For that, seminal samples were obtained from 10 male pigs (200µL, $2x10^6$spzt/mL). Data were analyzed using the PROC MIXED (SAS, v. 9.2 for Windows) adjusted by Tukey, comparing the lsmeans and analysis of interactions between groups. To determine the effects of PEI on seminal viability, the semen samples were incubated with 0.5 mg / mL PEI for 10 min or 2 h. The flow cytometry was used to evaluate the sperm viability (plasmatic membrane damage – PMD; acrosome damage – AD; DNA fragmentation – DNAF). The incubation for 2 h led to higher PMD index (66.46±2.70%, P<0.0001) in comparison to 10 min or control group (without PEI) (19.48±2.70% and 18.27±2.70%, respectively), but no difference was found for AD and DNAF among the groups. Then, the spermatozoon viability was established with a similar experiment but using the plasmid pmhyGENIE-5 complexed with PEI (400ng/mL, PEI/VET) and the transfection efficiency was determined by FISH. Additionally, the direct plasmid incubation without PEI was also carried out as a standard protocol. The direct plasmid incubation increased the DNAF (3.67±0.03%, P<0.001) in relation to the PEI/VET (0.81±0.16%) and control group (without transfection) (0.58±0.08%). On the other hand, the PEI/VET complex improved the PMD (P<0.001) in comparison to the incubation or control (66.97±3.55%; 20.60±3.81%; 21.91±3.55%, respectively), as well as the AD (70.79±3.68%; 19.49±3.61%; 31.68±3.61%, respectively). Despite the cell damage, the spermatozoa exposed to PEI/VET presented greater transfection index (76.80±3.09%, P<0.001) than the incubation (17.80±1.07%). These results suggest that the PEI could be an efficient and low-cost transfection method for swine sperm. It is worth to point out that the cells treated with PEI/VET showed higher indexes of PMD and AD, so that it would be interesting to combine it with bio-techniques that facilitate the fecundation (i.e. FIV or ICSI) or even inclusion of antioxidant or anti-apoptotic drugs to improve the spermatozoa viability.

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