Pig reproduction

PRODUCTION OF GENETICALLY MODIFIED PORCINE EMBRYOS BY LIPOFECTION OF IN VITRO MATURED OOCYTES USING CRISPR/CAS9 SYSTEM

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BACKGROUND-AIM

Lipofection has been commonly used to introduce external molecules into cells since its development in 1987 by Felgner et al (Proc Natl Acad Sci USA). However, its use in porcine oocytes and embryos to produce genetically modified pigs using CRISPR/Cas9 has not been studied in deep. As this technique can facilitate the procedure of producing genetically modified animals, as it does not need specific machinery or trained personal, we compare the use of lipofectamine versus electroporation in porcine oocytes to produce mutations in CD163 gene using the CRISPR/Cas9 system.

METHODS

In vitro matured oocytes were electroporated (E) or lipofected (L) with Lipofectamine CRISPRMAX Cas9 at two concentrations [5%(v/v) -1x group-, 10%(v/v) -2x group-] with sgRNA and protein Cas9, in vitro fertilized and in vitro cultured up to 6 days. A group without treatment was used as control. Cleavage and blastocyst rate (blastocyst/oocyte) were evaluated and mutation rates were analysed by fluorescent PCR-capillary gel electrophoresis. 328 oocytes were evaluated.

RESULTS

Cleavage rate was significantly lower in L2x group (18.3%, $p<0.01$) in comparison with control (41.7%), E (62.1%) and L1x groups (36.6%). Furthermore, the blastocyst rate was also significantly lower in L2x group (5.5%) in comparison with control group (16.7%, $p=0,03$), finding no differences between the others (E: 13.8%; L1x: 12.9%). These results suggest that a high concentration of lipofectamine can be toxic to the embryos, but no detrimental effect was detected with a low concentration. No significant differences were found between groups on mutation rate (E: 50%; L1x: 38.5%, L2x: 50%), mosaicism rate (E: 12.5%; L1x: 7.7%, L2x: 0%) and on the overall efficiency (E: 6.9%; L1x: 5%; L2x: 2.75%).

CONCLUSIONS

Previously, Hirata et al (Animals) used this method successfully in zona pellucida (ZP) free oocytes and embryos but the use of ZP intact oocytes is an advantage for embryo culture and transfer to recipients. For that reason and considering these results, lipofection of in vitro matured oocytes can be an effective alternative to produce genetically modified embryos and animals. However, an optimization of the conditions and more analysis are still needed.
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