

7. Summary

Production and improvement of virosomes for the use as vectors transferring transgenes into bovine sperm cells.

Generating transgenic livestock is the aim of many studies. To transfer foreign DNA into oocytes usually pronuclear microinjection is applied. Embryos extracted by this technique are transferred into foster mothers, but only 1% of transferred zygotes results in the birth of living, healthy calves. To improve the efficiency new methods have been developed, among others sperm mediated gene transfer (SMGT). By this technique sperm cells, incubated with DNA containing media, carry the transgene into the oocyte during fertilization.

Several methods have been published to transfer foreign genes into living cells. One method is to utilize the natural potential of viruses to transfer and replicate their genetic material into cells.

Here experiments are reported that were designed to exploit the fusion potential of reconstituted influenza virus envelopes (virosomes) for the transfer of foreign DNA into bovine spermatozoa. A protocol was developed for the preparation of virosomes bearing influenza X-31 hemagglutinin inserted into artificial lipid bilayers. To optimize virosomes for the sperm mediated transfer different steps during the reconstitution protocol were varied. Criteria for improving the virosomes were beside lipid, protein and plasmid content of virosomes also fusion and gene transfer efficiency to sperm cells.

Results of this study indicated that the protein inserted into the lipid bilayers consists mainly of influenza hemagglutinin. From the added lipids the major amount could be found in the virosomes. Interestingly adding about 6 mg cholesterol to a total amount of lipids from 25.15 mg seems to be necessary to promote fusion with cryopreserved bull spermatozoa. But the optimal lipid mixture seems to depend on the target cell. Additionally virosomes are able to fuse to sperm cells independent from individuum, developmental stage or species. Gene transfer to sperm cells varies from 1.5 to 302 plasmids per sperm cell.

Taken together this makes virosomes a promising tool for transferring foreign DNA into sperm cells of farm animals.