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The Cancer Research UK Beatson Institute, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD, Scotland United Kingdom.



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74. Using layered double hydroxides in ovine sperm-mediated gene transfer

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Layered double hydroxides (LDH) are chemical materials that can be synthesized from many different substances, have double lipid layers in their structures and it is highly important to determine their sizes, working pH values and synthesis methods in order to use them as transfection agents. When DNA molecules are being kept in between double lipid layers, it is aimed that it releases the DNA molecule in order to achieve a successful transfection in cells. LDH's are used in some cell transfection researches but they have not been used as a new vehicle in sperm-mediated gene transfer (SMGT) yet.

In our study, we used oocytes obtained from ovaries of slaughtered sheep and used two different kinds of LDH's Mg-Al-Cl-LDH and Ca-Al-Cl-LDH with the concentration of 2.5 ng/μL. pDNA (plasmid DNA) for the transfection of ram spermatozoa LDH's were mixed with DNA at 37 °C for 4 h, after that LDH-DNA complexes were incubated with spermatozoa for 1 h at room temperature. ICSI (intracytoplasmic sperm injection) was used to fertilize the MI ovine oocytes. We used EGFP (enhanced green fluorescent) gene to determine transgenesis under attempted with transfection. The controls of transgenic embryos are achieved according to the green luminescence status under fluorescent attached invert microscope. All the embryonic development procedures were made as in Birler et al. 2010, except we used only ionomycin activation and put oocytes in fertilization SOF media after ICSI.

Our results showed that in both Mg-Al-Cl-LDH and Ca-Al-Cl-LDH groups we obtained had transgenic embryos 13.04 % and 21.87 %, respectively, but not statistically important ($p > 0.05$). The cleavage ratios were 74.19 % and 71.11 % also the degeneration rates were 25.80 and 26.66, respectively, but not statistically important ($p > 0.05$). The embryonic development rates of the LDH groups were not lower than our control group.

In conclusion, we determined that both Mg-Al-Cl-LDH and Ca-Al-Cl-LDH can be used for SMGT in ovine transgenesis. They did not affect embryonic development negatively, and their transgenesis efficiency can be elevated with more researches in this field.

75. A vaginal pessary-based system for pseudopregnancy induction in laboratory mice

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Successful pseudopregnancy induction is key to implementing streamlined, effective protocols for the preparation of recipients for embryo transfer in transgenic/line rederivation-based embryo transfer programmes. This neuroendocrine induction hinges around a combination of vagina/cervical mechanical stimulation

by coitus and reproductive tract mucosal biochemical stimulation by seminal plasma. While good success rates are obtained by mating recipients with a vasectomised male, this approach has its own disadvantages, ranging from the ethical (increased animal usage, surgery) to the pragmatic (rest time/ardency-related variable success rates). The study therefore aimed to develop an alternative, male-free, vaginal pessary-based method for pseudopregnancy induction which artificially mimics natural mating.

Four-mm-diameter pedunculated nylon porous vaginal pessaries were created using selective laser sintering technology. Virgin 4- to 6-week-old CD-1, Whitten effect-synchronised females were randomly allocated to mating with a vasectomised male ($n = 25$) or to the use of pessary-based system with/without loading with a seminal plasma mimic ($n = 5$ and $n = 26$, respectively). Laparotomic unilateral oviductal embryo transfers were performed the following morning using 15 zygotes (obtained by mating superovulated donors to syngeneic stud males) per female. Recipients were individually housed on day 16 to litter down. Pregnancy rates, litter sizes, birth weights and pup fertility in adulthood were recorded. Data were analysed by C^2 analysis.

No pregnancies were achieved with unadulterated pessaries, while there was no significant difference in pregnancy rates (56 vs. 48 %; vasectomised male-mated and loaded pessary groups, respectively). Litter sizes and birth weights were comparable across these groups, and all pups were proven fertile when mated to syngeneic animals at 5 weeks.

This method offers a viable and ethically sound alternative to current vasectomised or sterile male mating-based approaches for pseudopregnancy induction. While the total replacement of males is of doubtless ethical value in reducing animal usage, it also has financial benefits (obviating the need to house additional animals) and pragmatic advantages (independence from male rest periods, no need to vasectomise/breed sterile males, more predictable numbers of pseudopregnant recipients available). This novel method therefore has the potential to improve workflow and reduce cost/dedicated staffing time, thus making embryo transfer more accessible to service users.

This method is protected by patent GB1314452.2.

76. RepLiCre: Reporter-linked-Cre transgenic mice for efficient use of conditional knockout mouse resources

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Many organizations worldwide have joined hands to establish large-scale programs such as IKMC (International Knockout