Genetic modification of bovine embryos by lentiviral vectors

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Lentiviral vectors have been widely used in studies for generation of human induced pluripotent stem cells (Okita and Yamanaka, 2011. Philos Trans R Soc Lond B Biol Sci 366:2198) and for human gene therapy (Antoniou et al, 2013. Hum Gene Ther 24:363). Such vectors have also been shown to be an alternative to generate livestock. As any other transgene delivery systems, lentiviral vectors have pros and cons. Its efficiency is elevated when compared to other systems. Lilico et al. (Trans Res 20:441, 2011) generated more transgenic lambs by lentiviral vectors in 2008/2009 (32 founders with 6 different transgenes) than the previous 25 years in the Roslin Institute using other techniques. The efficiency of lentiviral vector seems to be related to its nuclear import feature and ability to integrate into the genome of non-dividing cells (Durand and Cimareli, 2011. Viruses 3:132). However, the production and manipulation of these vectors require laboratories with biosafety level two, despite the third generation of lentiviral vectors has features that increases the biosafety and reduces undesirable effects as those caused by retrovirus, as activation of proto-oncogenes (Cockrell and Kafri, 2007. Mol Biotechnol 36:184). The transfer vector size, generally smaller than 13 kb, can be a limitation, allowing inserts with up to 7.5 kb only (Al Yacoub et al., 2007. J Gene Med 9:579). Moreover, expression of lentivirus integrants may be modulated by epigenetic modification and disturbs transgene expression (Hofmann et al., 2006. Mol Therap 13:59). The usefulness of lentiviral vectors to generate transgenic cattle was reported by Hofmann et al. (Biol Reprod 71:405, 2004) by microinjecting lentiviral particles into periviteline space of matured oocytes. Microinjection into periviteline space of bovine zygotes was shown to be less efficient than of oocytes (Hofmann et al, 2004. Biol Reprod 71:405; Ewerling et al., 2006 Transgenic Res 15:447). We have also carried out studies with lentiviral vectors to delivery GFP transgene to matured bovine oocytes and zygotes. Differently from previous studies, we microinjected lentiviral particles into the periviteline space of zygotes with 6h post in vitro fertilization in an attempt to make the transgene available before syngamy. Fifty percent of the blastocysts produced had the transgene detected by PCR in contrast to 100% of blastocyts produced from matured oocytes microinjected with lentiviral vectors. In both groups, the proportion of blastocysts emitting green fluorescence was lower than that of blastocyst with the transgene detected by PCR, suggesting the silencing of GFP expression in some embryos. Eleven blastocysts produced from matured oocytes microinjected with lentiviral vectors were transferred to synchronized recipients and resulted in five pregnancies (45.4%); rate similar to that regularly reported with non-microinjected vitro-fertilized embryos. However, one fetus was lost in the 8th month of pregnancy and two out four calves died few hours before parturition without any apparent morphological alteration. The transgene was detected by PCR in umbilical cord and blood cells from one of the stillborn calves while tissues from other three calves are still under evaluations. Those results indicate that the use of lentiviral vectors by microinjection into periviteline space of bovine oocytes and zygotes still demands improvements. Nevertheless, lentiviral vectors can also be used to transduce somatic donor cells in order to generate transgenic cloned animals and may be an alternative for production of transgenic cattle (Monzani et al. 2013. Gen Mol Res 12). Despite its potential application for cattle transgenesis, lentiviral vectors may become restrict to production of transgenic cows for secretion of recombinant biopharmaceutical proteins for human and animal health purposes. As the current lentiviral vectors are based on HIV-1 nucleotide sequences, the consumer may decline to consume milk or meat produced by cattle genetically modified by those vectors. Besides, new tools to edit the genome, as meganucleases, are becoming available for livestock and may have advantages over lentiviral vectors.

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