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## Pregnancy and birth of calves derived from in vitro-matured oocytes injected with lentiviral vector carrying GFP gene

L.S.A. CAMARGO[1], F.S. GONCALVES[1], M.M. PEREIRA[1], L.G.B. SIQUEIRA[1], J.R. TOLEDO[2], N.C. PARRA[2], C.C.R. QUINTAO[1] & J.H.M. VIANA[1]

*1-Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil; 2-Universidad de Concepcion, Concepcion, Chile*

Lentiviral vectors derived from human immunodeficiency virus (HIV) can be used to generate transgenic farm animals as pigs (Hofmann et al., 2003, EMBO Reports 4: 1054-1060) and sheep (Lillico et al., 2011, Transgenic Res 20:441-442). In cattle, lentiviral vectors can be associated to in vitro fertilization to generate in vitro-produced transgenic embryos (Hofmann et al., 2004, Biol Reprod, 71:405-409) as an alternative to nuclear transfer with transgenic somatic cells (SCNT), which efficiency in generating live animals is generally lower than 10% (Panarace et al., 2007, Theriogenology 67:142-151). In this study we aimed to evaluate the pregnancy and birth rates of in vitro-produced bovine blastocysts derived from in vitro-matured oocytes injected with lentiviral vector carrying GFP gene with a CMV promoter. Eleven blastocysts derived from oocytes injected with lentiviral vectors into perivitelline space were transferred to eleven recipients previously synchronized, and pregnancies were monitored by ultrasonography until birth. Five recipients became pregnant (45.4%; 5/11 embryos). From these five pregnancies, one ended in abortion in the 8$^{th}$ month of gestation and four were born. Two newborns died few hours after birth (18.2%; 2/11 embryos) without any apparent morphological abnormalities and two survived beyond six months (18.2%; 2/11 embryos). The overall mean gestation length was 272.5±5.7 days, with 268.0±7.0 days for those calves that survived and 277.0±1.4 days for those that died after birth. PCR analysis was carried out to detect the GFP gene in skin biopsies, umbilical cord and blood cells collected from the four newborns. Only one (25%; 1/4) newborn showed PCR product for GFP gene in all tissues and replicates analyzed, which is in agreement with our previous data that showed that 28.5% blastocysts were GFP positive under epifluorescence stereoscope (Camargo et al., 2014, Transgenic Res 23:204). We confirm that lentiviral vectors system can be used to generate calves with genetic modification; nevertheless, it still requires improvement to reduce calf losses after birth and increase the proportion of genetically engineered newborns.

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Palavras chave: animal reproduction, genetic, improvement, PIV



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