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Effect of lentiviral vectors microinjection into the perivitelline space of bovine early zygotes on embryo development and transgene expression

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Lentiviral vectors are an alternative to deliver transgenes into oocytes and zygotes. Previous study reported higher blastocyst production but lower expression rate when lentiviral vectors were microinjected in bovine zygotes after 18h of insemination and compared to matured oocytes (Ewerling et al., 2006, Transgenic Res 15:447-454). The present study evaluated embryo development and transgene expression after lentiviral microinjection into perivitelline space of early zygotes (6h post in vitro fertilization) or matured oocytes. In a preliminary experiment we evaluated the effect of short incubation period and of sperm concentration on embryo development. In vitro matured oocytes (n=591, five repetitions) were co-incubated with 2 or 4 million sperm/mL for 6 or 20h and cleavage and blastocyst rate were analyzed by Chi-square. Higher ($P<0.05$) cleavage rate was obtained with 4 million sperm for 20h whereas the blastocyst rate at day seven post-insemination was similar ($P<0.05$) between 4 million sperm for 6 and 20h. At day 8, the blastocyst rate was higher ($P<0.05$) for 2 and 4 million sperm for 20h (26.6% and 28.7%, respectively) than 2 and 4 million sperm for 6h (13.7% and 17.1%, respectively). Based on the preliminary results we evaluated the blastocyst rate and the expression of green fluorescent protein (GFP) transgene after perivitelline microinjection of lentiviral vectors in early zygotes (6h post in vitro fertilization) and in matured oocytes. Lentiviral particles were generated in 293T cells by co-transfection of pMDLg pRRE, pRSV Rev, pMD2.g (Addgene, Cambridge, MA, USA), and a modified pLGW with the GFP gene under the control of the CMV promoter. The supernatant was collected and ultra-centrifuged to concentrate the viral particles, which were microinjected into perivitelline space of oocytes (n=214, four repetitions) and early zygotes (n=138, four repetitions). GFP expression in blastocysts was visualized using an epi-fluorescence stereoscope with eGFP filter and GFP transgene incorporation was analyzed by PCR. Data were compared by Chi-square. There was no difference ($P>0.05$) in blastocyst rate (12.3% vs 13.1%) and proportion of embryos expressing GFP (29.4 vs 28.5%) between early zygotes and oocytes, respectively. However, PCR analysis revealed that 100% of the embryos generated from microinjected oocytes had the transgene, in contrast to 50% of those from early zygotes. Despite decreasing blastocyst production, short oocyte-sperm incubation period can be used to generate early zygotes for lentiviral perivitelline microinjection, but the efficiency of transgene incorporation is inferior to microinjected oocytes. Moreover, the difference between embryos emitting green fluorescence and embryos with the transgene incorporated suggests that silencing of GFP expression may occur at blastocyst stage. Financial support: CNPq 402607/2010-4 and FAPEMIG.

Speaker Abstracts

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TRANSGENIC ANIMAL RESEARCH CONFERENCE IX

August 11–15, 2013

Granlibakken Conference Center

Tahoe City, California