



A282E Cloning, Transgenesis and Stem Cells

Metabolomic analysis revealed differences between bovine cloned embryos with contrasting development abilities

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Cloning by somatic cell nuclear transfer has been established in various species but its efficiency remains low. Efforts have been made to improve cloning efficiency mainly focusing on reprogramming, using donor cells sourced from different somatic tissues and using epigenetic modifiers. Differences in reprogramming efficiency linked to the genotype of donor nuclei have been observed in mouse and bovine, but remained largely unexplained, especially in the bovine. This study is part of a set of analysis to understand the early differences between two types of bovine cloned blastocysts with different term development abilities (2.5 versus 12.5% birth rates) (Bui, *Reproduction*, 138(2), 289-99, 2009). Blastocysts were obtained by nuclear transfer of fibroblasts derived from ear skin of two different Holstein heifers (OV 0029 and OV5538) as described (Khan, *PlosOne*, 7(3), e34110, 2012). After activation, embryos were cultured in groups in SOF medium with 1% oestrus cow serum (39°C, 5%CO₂, 5%O₂). On Day 6 embryos were cultured individually for 24 hours in 12µl droplets of SOF (Minitube) plus Bovine Serum Albumin. On Day 7, 10µl spent culture medium (CM) and blank samples were collected and frozen until Fourier Transform Infrared Spectroscopy (FTIR) analysis. The metabolic fingerprint of spent CM of the two types of cloned blastocysts were compared. Briefly, samples (n=36 and 65, respectively for OV0029 and OV5538) were analyzed using a Golden-Gate ATR device mounted on a Varian 620-IR FTIR spectrophotometer. After correction for experimental series and subtraction of values obtained for blank samples from experimental values, the FTIR spectra were analyzed by redundancy analysis (RDA), a method which combines multiple regression with Principal Component Analysis. The model included embryo stage at day7, embryo grade at day7 (according to IETS grading; only grade 1 and 2 embryos) and embryo genotype. Significance of the effect "embryo genotype" was addressed with permutations tests. Analysis on the whole spectra did not show significant effects. However, focusing on sub-regions of the spectra pointed to a significant "embryo genotype" effect (p=0.035) for wavelengths between 2850 and 3030cm⁻¹. The above identified region of the spectra covers the CH₃ and CH₂ asymmetric stretching from lipids (Socrates, *Infrared and Raman Characteristic Group Frequencies, Tables and Charts*, Ed. Wiley, 2001 ch 23). Therefore, our results suggest differences in lipid metabolism between these two types of clones. Further analysis is in progress to confirm this hypothesis.

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Embryo collection in clone cattle offspring

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Our laboratory has been working on bovine clones for many years. These clones were studied and several cloned females were bred to obtain clone offsprings. All pregnancies were normal and calves developed as healthy individuals. The females were used for embryo collection after superovulation. The objective of this study was to compare the embryo recovery results between clone offspring and control animals. Altogether, 28 cows were used for this study (18 clone offspring and 10 controls). All the animals were born and raised in the same experimental farm, in the same time period and in the same rearing conditions. 90 flushes were performed to collect D9 to D21 embryos for research protocols. For early embryos on D9, a classical 3 way collection equipment (IMV, France) was used. To collect the late embryos D12-D21, the same equipment was modified so that larger embryos could be collected through the remaining larger hole (2 way collection) (Richard et al. 2015, *Theriogenology* 83, 1101-9). All females were submitted to ovum pick-up to remove the dominant follicle and were subsequently superovulated with FSH (Stimufol®, Reprobiol, Belgium). Luteolysis was induced 48 hours prior to AI. Two AI were performed with frozen semen, 48 and 56 hours after PGF2 α injection (Estrumate®, MSD Santé Animale, France). Before embryo collection, cows were treated with an epidural injection of 3-4 ml (Xylovet®, CEVA Santé Animale SA, France). The presence of Copora Lutea (CL) was checked and they were counted by rectal palpation. For all collections, the cervix was prepared with the initial introduction of a dilator. Then the catheter was introduced in one horn and the cuff was inflated as low as possible. For the collection of late stage embryos, 30 ml (Euroflush, IMV, France) was injected slowly twice to suspend the embryos prior to flushing the horn with 500 ml, and the same operation was performed on the second horn. Data were analyzed by unpaired t-test using Prim® software. There was no significant difference in the number of embryos collected per flush in clone offspring and controls (349 embryos collected, 5.05 ± 4.8 per flush vs 90 embryos collected, 4.28 ± 3.92 per flush, respectively). The number of CL was also not significantly different between groups (11.49 ± 7.32 and 8.43 ± 4.26 per flush, respectively). For late collections in all animals, the FSH dose (Stimufol®) was reduced to limit the number of embryos and preserve development (Richard et al. 2015). Retrospectively there was no significant difference for the necessary dose for superovulation (0.57 ± 0.08 for clone offspring and 0.54 ± 0.07 for controls). These data indicate that offspring of clones raised since birth in the same conditions as control heifers have the same ability to give embryos after superovulation treatment indicating equivalence of reproductive function.