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Denis Laloë, Daniel Le Bourhis, Vincent Brochard, A. Fernandez-Gonzales, Delphine Dube, et al.. Metabolomic analysis revealed differences between bovine cloned embryos with contrasting development abilities. 31. Colloque Scientifique de l'AETE, Association Européenne de Transfert Embryonnaire - European Embryo Transfer Association (AETE). FRA., Sep 2015, Ghent, Belgium. hal-01536465

**HAL Id: hal-01536465**

**<https://hal.science/hal-01536465>**

Submitted on 3 Jun 2020

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## 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%CO<sub>2</sub>, 5%O<sub>2</sub>). 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<sup>-1</sup>. The above identified region of the spectra covers the CH<sub>3</sub> and CH<sub>2</sub> 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.

*Supported by AGL2012-37772 and FEDER*