

***In vitro* development of cloned bovine embryos produced by handmade cloning using somatic cells from distinct levels of cell culture confluence**

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Genet. Mol. Res. 9 (1): 295-302 (2010)

Received September 14, 2009

Accepted November 16, 2009

Published February 18, 2010

ABSTRACT. The relationship between the level of cell confluence near the plateau phase of growth and blastocyst yield following somatic cell cloning is not well understood. We examined the effect of distinct cell culture confluence levels on *in vitro* development of cloned bovine embryos. *In vitro*-matured bovine oocytes were manually bisected and selected by DNA staining. One or two enucleated hemi-cytoplasts were paired and fused with an adult skin somatic cell. Cultured skin cells from an adult Nelore cow harvested at three distinct culture confluence levels (70-80, 80-90, and >95%) were used for construction of embryos

and hemi-embryos. After activation, structures were cultured *in vitro* as one embryo (1 x 100%) or as aggregates of two hemi-embryos (2 x 50%) per microwell. Fusion, cleavage and blastocyst rates were compared using the χ^2 test. The fusion rate for hemi-embryos (51.4%) was lower than for embryos (67.6%), with no influence of degree of cell confluence. However, blastocyst rates improved linearly (7.0, 17.5, and 29.4%) with increases in cell confluence. We conclude that degree of cell culture confluence significantly influences subsequent embryo development; use of a cell population in high confluence (>90%) for nuclear transfer significantly improved blastocyst yield after cloning.

Key words: Somatic cell nuclear transfer; Cell culture confluence; Cell cycle; Embryo aggregation; Cattle