Improved cloning efficiency using hCD55/alpha 1,3-Gal knock-out cell clones selected for high hCD55 expression level.

Andrea Perota\textsuperscript{1}, Irina Lagutina\textsuperscript{1}, Silvia Colleoni\textsuperscript{1}, Roberto Duchi\textsuperscript{1}, Giovanna Lazzari\textsuperscript{1}, Emanuele Cozzi\textsuperscript{2}, Fiorella Calabrese\textsuperscript{2}, Mathias Chatelais\textsuperscript{3}, Beatrice Charreau\textsuperscript{3}, Franco Lucchini\textsuperscript{4}, Cesare Galli\textsuperscript{1,5}

\textsuperscript{1}LTR-Avantea, Cremona, Italy; \textsuperscript{2}Dipartimento di Scienze Mediche e Chirurgiche, Ospedale di Padova, Padova, Italy; \textsuperscript{3}INSERM UMR 643, Institute de Transplantation et de la Recherche en Transplantation, Nantes, France; \textsuperscript{4}CRB-Università Cattolica del Sacro Cuore, Cremona, Italy; \textsuperscript{5}Dipartimento Scienze Mediche Veterinarie, Università di Bologna, Ozzano Emilia, Bologna, Italy

Although it was demonstrated that a-1,3 galactosyltransferase knock out (Gal-KO) pig organs transplanted into primates were protected from hyperacute rejection, hCD55 expression in donors is considered necessary to protect cells and tissues from complement-mediated damage. To provide sufficient number of cloned transgenic animals with high expression of CD55 for xenotransplantation, we combined intensive screening of colonies (ICC and WB) with
somatic cell nuclear transfer (SCNT). An outbred Gal-KO male minipig line was established and nucleofected with a strong ubiquitous expression vector (pCAGGS-hCD55). Hygromicine resistant clones (n=10) were selected for hCD55 expression (WB, ICC) using the mAb IA10 (BD-Pharmingen). The 8 best clones were used for SCNT - 4 clones/experiment. In total 899 morula and blastocysts D6 were transferred into 11 synchronized sows: 7 became pregnant (64%); 4 went to term, 1 aborted and 2 are ongoing. Twenty nine piglets were born (15 alive and 14 stillborn) and 12 were live at 7\textsuperscript{th} day (80%). At birth umbilical cord samples (UCS) from cloned animals were used for phenotypic (WB, ICC) and genotypic characterizations (PCR and Southern blot). WB detected the hCD55 expression in every UCS and tissue from stillborn pigs (n = 7). ICC confirmed high level of hCD55 expression in primary fibroblasts (n=5), kidney cells (n=7) and on PAEC (n=5). After Southern blot new 4 different hCD55/a1,3-GalKO minipig cell lines were identified. Thus the selection of the fibroblasts prior to SCNT is very effective to ensure the desired expression in the animals, moreover pooling of 4 different clonal fibroblasts increased the birth rate since only 4 out of the 8 clones generated offsprings.

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