Adipose mesenchymal stem cells: a new tool to restore interesting genotypes by cloning in the rabbit

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Our objective was to investigate somatic cell nuclear transfer (SCNT) as a tool for restoration of particular genotypes (genome edited mainly) in the New-Zealand rabbit. SCNT efficiency is founded on the capacity of donor cells to be reprogrammed to a totipotent state. Consequently, the less differentiated donor cells are, the more easily they could be reprogrammed by a recipient ooplasm. In rabbit, the lack of functional embryonic stem cells is thus a problem. In Ali/Bas rabbit, V. Zakhartchenko *et al.* (Biol Reprod.84p229. 2011) opened interesting perspectives with the use of bone marrow multipotent cells as donor cells for SCNT. Thus, multipotent mesenchymal stem cells (MSC) could be attractive for our purpose. From this prerequisite but looking for multipotent cells accessible in the least invasive way for the donor rabbit, we tested the ability of adipose-derived mesenchymal stem cells (ASC) to give birth to cloned animals. ASC were easily recovered from abdominal fat under anaesthesia. For this preliminary study, we used 2 different batches of commercial ASC (RBXMD-01001/Cyagen Biosciences, Neu-Isenburg, Germany) chosen for their multipotent state and strong capacity to expand maintaining this state. We used cumulus cells (CC) as "control" of development potential since they have been used widely for SCNT and most rabbit live clones were produced from freshly prepared CC. Nuclear transfer and embryo transfer were performed as described by N. Daniel *et al.* (Methods Mol Biol.1222p15. 2015 and Cold Spring Harb Protoc. 2010). The pregnancies were followed by ultrasound monitoring as described by P. Chavatte-Palmer *et al*. (Theriogenology.69p859. 2008). *In vitro* and *in vivo* embryo developments were compared by Chi-2 or non-parametric Fisher's exact test and differences were considered significant at $P < 0.05$. We first compared 2 ASC lines to make sure that the individual characteristics of each do not influence the developmental competence of SCNT embryos. No significant differences were observed for cleavage, blastocyst, implantation and pregnancy rates, nor for development to term. We then compared ASC versus (vs) CC. ASC showed higher *in vitro* development rates: 88% (492/559) vs 73.5% (180/245) and 46.1% (65/141) vs 32.2% (79/245) for cleavage and blastocyst rates respectively. At mid-gestation, pregnancy rates were not significantly different: 40.1% (9/22) vs 50% (4/8). Term pregnancies were obtained for 1 and 3 recipient females respectively. One clone was born from ASC and 5 from CC. Embryo competence to develop to term was thus significantly lower for ASC 0.4% (1/247) vs 3.6% (5/138). Large Offspring Syndrome was observed for 1 ASC and 2CC clones. Further studies are thus necessary to decrease LOS incidence in rabbit cloning, but our study showed that ASC, which are easily available for multiple cloning sessions, are compatible with full term pregnancy after SCNT.

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