Advances in the generation of multi-transgenic pigs by somatic nuclear transfer cloning

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Somatic nuclear transfer involves five major steps, including enucleation, transfer of the donor cell, fusion, activation and culture of the reconstructed embryo. Enucleation is usually performed by sucking out a small portion of the oocyte's cytoplasm containing the maternal chromosomes into a small bevelled pipette. To avoid damage of the cytoplasm, the oocyte is treated with a cytoskeleton inhibitor, mostly cytochalasin B. The donor cell is usually derived from primary cell cultures of various tissue. Fibroblasts are frequently employed as donor cells. In the second step of nuclear transfer, a single donor cell is inserted under the zona pellucida as close as possible to the oocyte membrane. Both components are subsequently fused using short, but high voltage pulses at right angles to the juxtaposition of the two cells. Reconstructed embryos are activated either by electrical pulses and/or temporary exposure to chemical substances regulating the calcium influx and /or the cell cycle. The final step is the culture in vitro to the desired stage of development before cloned embryos are transferred to foster mothers. Viable clones have been produced in eleven mammalian species until today. Cloning technology is still characterized by low success rates and abnormalities frequently observed in the cloned offspring known as the "Large Offspring-Syndrome" (LOS). Only in cattle success rates can reach 15-20% viable offspring from cloned embryos, in all other species success rates are 1-3%.

Somatic nuclear transfer holds great promise for significant improvements in the generation of transgenic livestock. It has been shown that donor cells can be successfully transfected with different types of gene constructs and viable cloned transgenic offspring with stable integration have been obtained in sheep, cattle, goats, and pigs. The main advantage is the possibility of selecting the donor cells for optimal integration and expression of the transgenic construct and their direct use in nuclear transfer as well as the possibility of targeted genetic modifications. Most groups interested in large transgenic animals have therefore switched from microinjection to nuclear transfer for the production of transgenic livestock. Due to important modifications of the cloning protocol, we can now routinely produce cloned transgenic pigs with high levels of efficiency.

The hyperacute rejection response (HAR) which was the premier hurdle in porcine–to-human xenotransplantation, can already be overcome in a clinically relevant manner by expression of human complement regulatory proteins in transgenic pigs. However, despite severe immunosuppressive treatment the acute vascular rejection (AVR) with the disseminated intravascular coagulation (DIC) as the preeminent feature and the cellular rejection remain major obstacles for long term survival of a porcine xenograft. DIC is frequently observed in a pig-to-primate xenotransplant model and is caused by activation of the endothelial cells mainly attributed to incompatibilities between human and porcine coagulation factors. The goal of current research in the author`s laboratory is the generation and characterization of improved lines of multi-transgenic pigs targeting the AVR and specifically this coagulation disorder. We have produced and partially characterized transgenic pigs expressing constructs for the human complement regulators CD 55, CD 59 and thrombomodulin (hTM). The generation of multi-transgenic pigs with high expression of the various molecules will significantly improve long-term survival of porcine xenografts and will be a major step forward in the clinical application of xenotransplantation.

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