Animals as a source of organs and tissues for xenotransplantation could become a backup solution for the growing shortage of human donors. The presence of human xenoreactive antibodies directed against Galα1,3Gal antigens on the cell surface of a pig donor triggers the activation of the complement leading to a hyperacute reaction. The development of genetic engineering techniques has enabled the modification of genomes by knocking in and/or knocking out genes. In this paper, we report the generation of modified pigs with ZFN mediated disruption of the GGTA1 gene encoding the enzyme responsible for synthesis of Galα1,3Gal antigens.

ZFN plasmids designed to target the exon 9 region of the pig GGTA1 gene encoding the catalytic domain were injected into the pronuclei of fertilized egg cells. Among 107 piglets of the F0 generation analyzed, one female with 9-nt deletion in exon 9 of the GGTA1 gene was found. 13 of 33 piglets of the F1 generation represented the +/− GGTA1 genotype and 2 of 13 F2 piglets represented the −/− GGTA1 genotype. No changes in the animals’ behavior, phenotype or karyotype were observed. Analysis confirmed heredity of the trait in all animals. A complex functional analysis of the modified animals, including flow cytometry, human serum cytotoxicity test and immunohistochemical detection, was performed to estimate the phenotype effect of genetic modification and this indicated an efficient GGTA1 knock-out in modified pigs.

Key words: Galα1,3Gal epitopes, pigs, xenotransplantation, genome edition, ZFNs, functional characteristics